VNIVERSITAT DE VALÈNCIA

Programa de Doctorado en Enfermería Clínica y Comunitaria

Exposición prenatal a manganeso y a arsénico y efectos en el desarrollo neuropsicológico en niños y niñas participantes en la cohorte INMA

Tesis doctoral con Mención Internacional de Doctor

Raquel Soler Blasco

Dirección de tesis:

Dr. Ferran Ballester Díez

Dra. Sabrina Llop Pérez

Dr. Mario Murcia Hinarejos

Valencia, febrero, 2022

Ferran Ballester Diez, Doctor en Medicina, Catedrático de Universidad, Área de Enfermería, Universitat de València; M. Sabrina Llop Pérez, Doctora en Salud Pública y Mario Murcia Hinarejos, Doctor en Salud Pública

CERTIFICAN

Que Raquel Soler Blasco, Diplomada en Enfermería por la Universitat de València, y Máster en Salud Pública y Gestión Sanitaria por la Universitat de Valencia, ha realizado su tesis doctoral bajo nuestra dirección con el título de "*Exposición prenatal a manganeso y a arsénico y efectos en el desarrollo neuropsicológico en niños y niñas participantes en la cohorte INMA*".

Una vez revisado el presente trabajo, consideramos que reúne las condiciones para ser presentado y defendido como TESIS DOCTORAL.

Y para que conste a los efectos oportunos firmamos el presente certificado.

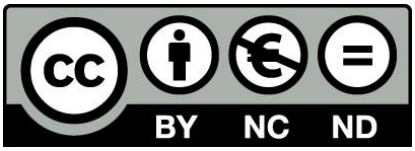
En Valencia, a 10 de febrero de 2022

Fdo:

Dr. Ferran Ballester Diez Dra. Sabrina Llop Pérez Dr. Mario Murcia Hinarejos

La presente Tesis Doctoral ha sido realizado gracias a la Ayuda Predoctoral de Formación en Investigación en Salud (PFIS, con referencia 17/00260), así como a las subvenciones concedidas al proyecto INMA (Infancia y Medio Ambiente. www.proyectoinma.org).

Una parte de este trabajo fue presentada como comunicación oral en el Congreso Virtual de la Sociedad Española de Epidemiología (SEE), y de la Associação Portuguesa de Epidemiologia (APE), en octubre de 2020. En ella, ésta fue premiada como una de las diez mejores comunicaciones presentadas por investigadores jóvenes (premio SEE-CIBERESP)



Un país sin investigación es un país sin desarrollo.

Margarita Salas

La ciencia siempre vale la pena, porque sus descubrimientos,

tarde o temprano, siempre se aplican.

Severo Ochoa

We should act together to create healthier, cleaner, and safe environments for children, which will contribute to a more secure future for the world. Therefore, the accumulation of the scientific knowledge and reinforcement of political actions based on scientific evidence are important

En Kishi R, Grandjean P (2019)

AGRADECIMIENTOS

Allá por 2017, mientras terminaba el Máster de Salud Pública, ya sabía que quería realizar el doctorado. Aspiraba y soñaba a poder trabajar como investigadora. Sin embargo, llegar hasta ahí lo veía prácticamente imposible. Pero un correo que recibimos todos los alumnos de un profesor del máster (mi primer agradecimiento: gracias, Ferran) sobre la posibilidad de hacer el doctorado con un contrato predoctoral cambió totalmente el rumbo del ese "*prácticamente imposible*". Y aquí estamos.

Durante estos cuatro años, quienes me conozcan me habrán escuchado decir en muchísimas ocasiones lo profundamente agradecida que estoy por haber tenido la suerte de poder estar dónde estoy ahora, y lo feliz que me hace trabajar dónde lo hago.

Los primeros agradecimientos son para mis directores y directora de tesis. Muchísimas gracias, Ferran, Sabrina y Mario. Primero, por la confianza depositada en mí. Me distéis la oportunidad de trabajar con vosotros, y gracias a eso he podido aprender de unos profesionales (y personas) excepcionales. Me habéis enseñado a ser independiente, a pensar con coherencia y cuidado en cada una de las etapas de investigación, a ver los resultados en un contexto práctico y me habéis animado a discurrir siempre un poquito más allá. Todo esto, y mucho más, lo habéis hecho con paciencia, respeto, profesionalidad e ilusión (esos "*dale, dale al clic*" cuando hemos enviado los artículos son puro oro).

El despacho. Ay, el despacho. El ambiente familiar que se respira allí es inmejorable. Siempre, absolutamente siempre, hay alguien que te echa un cable en cuanto lo necesitas, en lo que sea. Llúcia, Amparo C, Amparo F, Marisa, Rima, Gabi, Manu, Michele, Paula, Blanca, Tatiana, Natalia, M José, Christian, Olga, Rubén, Andrea…os doy las gracias a todos y todas, por hacerme sentir en mi segunda casa, por las charlas geniales en las comidas y en el descanso del café (no todo tiene que ser ciencia), por el humor y las risas incluso en momentos de presión, y por otros cientos de cosas más.

Muchas gracias también a otros investigadores e investigadoras del Proyecto INMA, especialmente al grupo de Gipuzkoa, tanto por permitirme usar parte de su trabajo para esta tesis, como por su apoyo desde la distancia.

Mi especial agradecimiento a las familias INMA. Estar en este proyecto me ha permitido valorar el esfuerzo que hacen los y las participantes de un estudio, especialmente en este. Sin ellos, no tendríamos nada, ni nosotros como investigadores, ni la sociedad en general. Sigo manteniendo que se les debería hacer un monumento.

Y ahora me gustaría dar las gracias a mi familia. A mi padre y a mi madre, que siempre (y siempre es siempre) me han apoyado en todo. Se han ilusionado por cada artículo, congreso, charla que he hecho, han escuchado pacientemente mis tejemanejes científicos y se han esforzado por entender todo este mundo, haciéndome sentir cuidada y apoyada. No tengo palabras para agradecéroslo. Gracias a mi hermana, por ser como es, por escucharme, cuidarme y hacerme reír, y por regalarme a Sofía y Leo. Espero con ganas más "*Tata, vamos a jugar a ciencia*". Y por supuesto, a Paco y a Tina, por su compañía infinita sin (casi) pedir nada a cambio.

Finalmente, quiero dar las gracias a mi otra familia. A mis *Inglourious* y al resto de mis amigos y amigas. Gracias por ser cómo sois. No puedo agradecer lo suficiente la suerte que he tenido en encontraros. Me habéis hecho ser más curiosa y crítica, hemos creado un "bebé" (ahora ya un chiquillo grande) cuyo late motiv es "la ciencia puede ser divertida" (y tanto), y siempre habéis estado ahí, para poder compartir cualquier pena y alegría, cualquier bajón o subidón, o simplemente un ratico bueno.

Gracias a todos y todas por estar ahí :)

PRÓLOGO

Esta tesis doctoral se presenta como compendio de tres publicaciones, en el marco del programa de doctorado de Enfermería Clínica y Comunitaria de la Universitat de València. El objetivo general de la tesis es evaluar la exposición prenatal a manganeso y a arsénico y sus diferentes especies, así como analizar la eficiencia en la metilación del arsénico en el embarazo. También, se pretende estudiar si dicha exposición prenatal se asocia a efectos en el desarrollo neuropsicológico evaluado durante la infancia.

Para la consecución de estos objetivos, el trabajo de tesis doctoral se enmarca en el Proyecto Infancia y Medio Ambiente (Proyecto INMA). Este estudio multicéntrico de cohortes, iniciado en el año 2003, tiene como principal objetivo estudiar el papel de los contaminantes ambientales más relevantes en el aire, el agua y la dieta durante el embarazo y el inicio de la vida, y sus efectos en el crecimiento y desarrollo infantil.

Mi participación en el Proyecto INMA comenzó en el año 2018, cuando recibí una Ayuda Predoctoral para Formación de personal en Investigación en Salud (PFIS, referencia 17/00260) del Instituto de Salud Carlos III de Madrid. Durante el primer año, y mientras esperábamos los datos de especiación del arsénico en orina durante el embarazo, comencé a familiarizarme con la información derivada de las diferentes visitas de seguimiento del proyecto, así como la revisión de la literatura sobre exposición prenatal a arsénico y efectos en el neurodesarrollo. Debido a un retraso en el análisis de las muestras, durante el primer y el segundo año mis directores y directora de tesis sugirieron estudiar los efectos de la exposición prenatal a manganeso sobre el neurodesarrollo infantil. Esta investigación culminó con la publicación del artículo "*Prenatal manganese exposure and neuropsychological development in early childhood in the INMA cohort*" (artículo I). Compaginando este trabajo, durante el segundo, tercer y cuarto año pude llevar a cabo los otros dos estudios que componen la presente tesis doctoral. En el primero de ellos, titulado "*Urinary arsenic species and methylation efficiency during pregnancy: concentrations and associated factors in Spanish pregnant women*" (artículo II) se evaluó la exposición a arsénico y sus diferentes metabolitos, así como la eficiencia de metilación durante el primer trimestre del embarazo, y se identificaron los factores asociados a estas variables. En último trabajo, publicado durante el año 2022 y titulado "*Prenatal arsenic exposure, arsenic methylation efficiency, and neuropsychological development among preschool children in a Spanish birth cohort*" (artículo III), pudimos evaluar la relación entre la exposición prenatal a arsénico y el desarrollo neuropsicológico a los 4-5 años de edad, identificando el efecto específico de cada una de las especies de As analizadas en la orina materna. Esto último ha supuesto un hecho novedoso, ya que no se han identificado estudios previos que hayan analizado esta relación.

Durante el último año de la tesis, he podido realizar una estancia predoctoral durante tres meses en la Unidad de Medicina Laboral y Ambiental, de la Escuela de Salud Pública y Medicina Comunitaria de la Universidad de Gotemburgo, bajo la supervisión de la profesora Florencia Harari. El objetivo principal de la estancia de investigación ha sido explorar la influencia de factores genéticos maternos y del niño o niña en el metabolismo del arsénico y en la aparición de efectos adversos en el desarrollo neuropsicológico infantil. Los resultados del presente estudio son todavía preliminares y se prevé seguir con esta línea de investigación en posteriores etapas.

Gracias al desarrollo de mi proyecto de tesis he podido obtener experiencia y desarrollar habilidades en investigación en epidemiología y salud pública, específicamente en el estudio de la exposición temprana a metales, tanto tóxicos como esenciales, y su efecto en el desarrollo neuropsicológico infantil, desempeñando tareas de revisión bibliográfica, concepción metodológica, análisis estadístico de los datos, interpretación de los resultados y redacción de manuscritos. Además, durante este periodo he podido presentar los resultados de estas investigaciones en diferentes congresos, tanto nacionales como internacionales. Finalmente, durante estos años he participado activamente como trabajadora de campo en la recogida de datos de la visita de seguimiento de los 14-16 años de la cohorte de Valencia del Proyecto INMA.

Esta actividad la he desarrollado en el Área de Ambiente y Salud de la Fundación para la Investigación Sanitaria y Biomédica de la Comunidad Valenciana (FISABIO), formando parte de la Unidad Mixta de Investigación en Epidemiología, Ambiente y Salud, entre la Universitat de València, Universitat Jaume I de Castellón y FISABIO, así como del grupo de investigación METALIA (METALs and chIdren's heAlth), liderado por una de mis directoras de tesis, la Dra. Sabrina Llop.

La presente memoria se presenta según las directrices del "*Reglamento sobre depósito, evaluación y defensa de la tesis doctoral*" de la Universitat de València y reúne los requisitos para ser presentada como tesis doctoral por compendio de publicaciones. Finalmente, parte del trabajo se presenta en lengua inglesa (Abstract and Conclusions) como requerimiento para obtener la Mención Internacional de doctor.

Nota sobre el uso de lenguaje igualitario:

El uso de lenguaje no discriminatorio y la sensibilidad y adecuación de los textos son una necesidad hoy en día. Para la consecución de esta tesis se ha tratado de seguir las recomendaciones propuestas por la "*Guía de uso para un lenguaje igualitario*" de la Universidad de Valencia (2012), en aras de encontrar un equilibrio entre el respeto por la igualdad de género y la necesidad de precisión y claridad del texto. Para ello se ha priorizado el uso de términos colectivos (por ejemplo, "infancia"), y de desdoblamientos ("los niños y niñas") en algunas ocasiones. No obstante, con el fin de asegurar la fluidez lectora que requiere la lectura un manuscrito de tesis doctoral, se utiliza el género neutro (niños) para hacer referencia a ambos sexos.

RESUMEN

Introducción: El manganeso (Mn) es un elemento esencial, indispensable para el desarrollo durante la etapa fetal. Por su parte, el arsénico (As) es un tóxico para el ser humano, siendo su forma inorgánica (iAs) la considerada más tóxica. La principal vía de exposición a ambos compuestos en población general es a través de la dieta. Una vez es absorbido, el iAs es biotransformado en ácido monometilarsónico (MMA) y ácido dimetilarsínico (DMA). Diversos estudios epidemiológicos han relacionado la exposición prenatal a estos metales con efectos adversos en el desarrollo neuropsicológico durante la infancia, aunque la evidencia actual no es concluyente. Además, la mayoría de estos estudios se han realizado en áreas con una alta exposición ambiental a estos compuestos, por lo que la evidencia en zonas con menores niveles en el ambiente, incluyendo agua y alimentos, es todavía escasa. El **objetivo general** de la presente tesis es estudiar la relación entre la exposición prenatal a Mn y a As y la aparición de efectos adversos en el desarrollo neuropsicológico infantil en los participantes de dos cohortes materno-infantiles, así como evaluar los factores asociados a dichas exposiciones.

Metodología: este trabajo forma parte del proyecto multicéntrico de cohortes Infancia y Medio Ambiente (INMA). La población de estudio fue entre 807 y 1179 pares madre-hijo/a de las cohortes de Gipuzkoa y Valencia (2004-2008). Durante el primer trimestre del embarazo se recogieron muestras de suero y orina en las que se analizaron las concentraciones de Mn y As total (TAs) y sus metabolitos (MMA, DMA, As inorgánicos [iAs] y arsenobetaína [AB]), respectivamente. El desarrollo neuropsicológico de los niños y niñas se evaluó mediante las Escalas de Desarrollo Infantil de Bayley (BSID) al año de edad y a través de las Escalas de Habilidades Infantiles McCarthy (MSCA) a los 4-5 años de edad. Se recogió información sociodemográfica, de estilos de vida y dietética mediante cuestionarios, así como otros biomarcadores maternos (ferritina sérica y zinc (Zn) urinario, entre otros). La eficiencia de metilación del As se determinó a través de los porcentajes de los metabolitos y utilizando el análisis de componentes principales. Se utilizaron modelos de regresión lineal multivariante para evaluar los factores asociados a las variables de exposición, así como para evaluar la relación entre la exposición prenatal a Mn, a As y sus metabolitos, así como la eficiencia en la metilación del As y las puntuaciones en las pruebas neuropsicológicas. Finalmente se examinó la linealidad de las relaciones y se exploró la posible modificación del efecto relacionada con diversas variables.

Resultados: la media geométrica (MG) e intervalo de confianza del 95% (IC95%) de las concentraciones de Mn en suero durante el primer trimestre del embarazo fue de 1,50 µg/L (1,48-1,53). El único factor dietético asociado a estas concentraciones fue el consumo de frutos secos. Las madres trabajadoras presentaron menores concentraciones de Mn en suero. Por su

parte, la MG (IC95%) de las concentraciones de TAs, AB, ∑As (suma de DMA, MMA e iAs), DMA, MMA e iAs en orina fue de 35,55 (33,10-38,19), 20,17 (18,34-22,19), 7,74 (7,41-8,09), 6,82 (6,52-7,14), 0,34 (0,32-0,36) y 0,33 (0,31-0,35) μg/g creatinina, respectivamente. El consumo de arroz se asoció de forma directa con las concentraciones urinarias de todos los metabolitos de As, excepto con la AB. De la misma manera, el consumo de pescado se asoció positivamente con las concentraciones de todos los metabolitos evaluados excepto con el MMA y el iAs. Otros factores asociados con las concentraciones de ciertas especies de As fueron el área de estudio, el país de origen de la madre, la clase social parental y el índice de masa corporal materno (IMC). Respecto a la eficiencia en la metilación del As durante el embarazo, las medianas (IC del 95%) del porcentaje de metabolitos de As fueron 89,7 (89,3-90,2) para %DMA, 5,1 (4,8-5,3) para %MMA y 4,7 (4,5-5,0) para %iAs. Las mujeres nacidas en Latinoamérica y aquellas con un mayor IMC presentaron una mejor metilación del iAs (indicado por mayor %DMA y menor %MMA y %iAs). En este estudio de cohortes multicéntrico no se encontró asociación entre la exposición prenatal a Mn y el desarrollo mental (β [IC95%] = −0,39 [−2,73 a 1,95]), ni con el desarrollo psicomotor (β [IC95%] = −0.92 [−3,48 a 1,65]) de los niños y niñas al año de edad. Respecto a la exposición prenatal a As, en el presente trabajo se observó una relación negativa entre las concentraciones urinarias de MMA durante el primer trimestre del embarazo y las puntuaciones de las escalas general, verbal, cuantitativa, de memoria, de función ejecutiva y de memoria de trabajo del MSCA a los 4-5 años de edad (por ejemplo, β [IC95%] = −1,37 [−2,33 a −0,41] para la escala general). Además, el %MMA se asoció de manera negativa con la subescala de memoria. Finalmente, se observó que los niños y niñas cuyas madres presentaron concentraciones más bajas de Mn, Zn y ferritina obtuvieron puntuaciones más bajas en varias escalas de MSCA con una eficiencia de metilación decreciente.

Conclusiones: Los niveles de Mn, As y sus metabolitos analizados en nuestra población son más bajos que los observados en la mayoría de los estudios publicados. Ciertos factores dietéticos, de estilo de vida y ambientales influyen tanto en las concentraciones de Mn y de especies de As, así como en la eficiencia de metilación del As en nuestra población. Este estudio no muestra asociación entre los niveles maternos prenatales de Mn y el desarrollo neuropsicológico al año del nacimiento. No obstante, sí se ha observado una asociación inversa entre las concentraciones de MMA y el desarrollo neuropsicológico de los niños y niñas a los 4-5 años de edad. Además, los niveles maternos de Mn, Zn y ferritina modificaron la asociación entre la eficiencia de metilación del As y las puntuaciones de MSCA.

RESUM

Introducció: El manganés (Mn) és un element essencial, indispensable per al desenvolupament durant l'etapa fetal. Per la seua part, l'arsènic (As) és un tòxic per a l'ésser humà, sent la seua forma inorgànica (iAs) la considerada més tòxica. La principal via d'exposició a tots dos compostos en població general és a través de la dieta. Una vegada és absorbit, el iAs és biotransformat en àcid monometilarsónic (MMA) i àcid dimetilarsínic (DMA). Diversos estudis epidemiològics han relacionat l'exposició prenatal a aquests metalls amb efectes adversos en el desenvolupament neuropsicològic durant la infància, encara que l'evidència actual no és concloent. A més, la majoria d'aquests estudis s'han realitzat en àrees amb una alta exposició ambiental a aquests compostos, per la qual cosa l'evidència en zones amb menors nivells en l'ambient, incloent aigua i aliments, és encara escassa. L'**objectiu general** de la present tesi és estudiar la relació entre l'exposició prenatal a Mn i a As i l'aparició d'efectes adversos en el desenvolupament neuropsicològic infantil en els participants de dues cohorts matern-infantils, així com avaluar els factors associats a aquestes exposicions.

Metodologia: aquest treball forma part del projecte multicèntric de cohorts Infància i Medi Ambient (INMA). La població d'estudi va ser entre 807 i 1179 parelles mare-fill/a de les cohorts de Guipúscoa i València (2004-2008). Durant el primer trimestre de l'embaràs es van recollir mostres de sèrum i orina en les quals es van analitzar les concentracions de Mn i As total (TAs) i els seus metabòlits (MMA, DMA, As inorgànic [iAs] i arsenobetaína [AB]), respectivament. El desenvolupament neuropsicològic dels xiquets i xiquetes es va avaluar mitjançant les Escales de Desenvolupament Infantil de Bayley (BSID) a l'any d'edat i a través de les Escales d'Habilitats Infantils McCarthy (MCSA) als 4-5 anys. Es va recollir informació sociodemogràfica, d'estils de vida i dietètica mitjançant qüestionaris, així com uns altres biomarcadors materns (ferritina sèrica i zinc (Zn) urinari, entre altres). L'eficiència de la metilació de l'As es va determinar a través dels percentatges dels metabòlits i utilitzant l'anàlisi de components principals. Es van utilitzar models de regressió lineal multivariant per a avaluar els factors associats a les variables d'exposició, així com per a avaluar la relació entre l'exposició prenatal a Mn, a As i els seus metabòlits, així com l'eficiència de la metilació de l'As i les puntuacions en les proves neuropsicològiques. Finalment es va examinar la linealitat de les relacions i es va explorar la possible modificació de l'efecte relacionada amb diverses variables.

Resultats: la mitjana geomètrica (MG) i interval de confiança del 95% (IC95%) de les concentracions de Mn en sèrum durant el primer trimestre de l'embaràs va ser de 1,50 µg/L (1,48-1,53). L'únic factor dietètic associat a aquestes concentracions va ser el consum de fruits secs. Les mares treballadores van presentar menors concentracions de Mn en sèrum. Per part seua, la

MG (IC95%) de les concentracions de TAs, AB, ∑As (suma de DMA, MMA i iAs), DMA, MMA i iAs en orina va ser de 35,55 (33,10-38,19), 20,17 (18,34-22,19), 7,74 (7,41-8,09), 6, 82 (6,52-7,14), 0,34 (0,32-0,36) i 0,33 (0,31-0,35) µg/g creatinina, respectivament. El consum d'arròs es va associar de manera directa amb les concentracions urinàries de tots els metabòlits d'As, excepte amb la AB. De la mateixa manera, el consum de peix es va associar positivament amb les concentracions de tots els metabòlits avaluats excepte amb el MMA i el iAs. Altres factors associats amb les concentracions de certes espècies d'As va ser l'àrea d'estudi, el país d'origen de la mare, la classe social parental i l'índex de massa corporal matern (IMC). Respecte a l'eficiència de la metilació de l'As durant l'embaràs, les mitjanes (IC del 95%) del percentatge de metabòlits d'As van ser 89,7 (89,3-90,2) per a %DMA, 5,1 (4,8-5,3) per a %MMA i 4,7 (4,5-5,0) per a %iAs. Les dones nascudes a Llatinoamèrica i aquelles amb un major IMC van presentar una millor metilació del iAs (indicat per major %DMA i menor %MMA i %iAs). En aquest estudi de cohorts multicèntric no es va trobar associació entre l'exposició prenatal a Mn i el desenvolupament mental (β [IC95%] = −0,39 [-2,73 a 1.95]), ni amb el desenvolupament psicomotor (β [IC95%] = −0.92 [-3,48 a 1,65]) dels xiquets i xiquetes a l'any d'edat. Respecte a l'exposició prenatal a As, en el present treball es va observar una relació negativa entre les concentracions urinàries de MMA durant el primer trimestre de l'embaràs i les puntuacions de les escales general, verbal, quantitativa, de memòria, de funció executiva i de memòria de treball del MSCA als 4-5 anys (per exemple, β [IC95%] = −1,37 [-2,33 a −0,41] per a l'escala general). A més, el %MMA es va associar de manera negativa amb la subescala de memòria. Finalment, es va observar que els xiquets i xiquetes les mares dels van presentar concentracions més baixes de Mn, Zn i ferritina van obtindre puntuacions més baixes en diverses escales de MSCA amb una eficiència de metilació decreixent.

Conclusions: Els nivells de Mn, As i els seus metabòlits analitzats en la nostra població són més baixos que els observats en la majoria dels estudis publicats. Certs factors dietètics, d'estil de vida i ambientals influeixen tant en les concentracions de Mn i d'espècies d'As, així com en l'eficiència de metilació de l'As en la nostra població. Aquest estudi no mostra associació entre els nivells materns prenatals de Mn i el desenvolupament neuropsicològic a l'any del naixement. No obstant això, sí s'ha observat una associació inversa entre les concentracions de MMA i el desenvolupament neuropsicològic dels xiquets als 4-5 anys d'edat. A més, els nivells materns de Mn, Zn i ferritina van modificar l'associació entre l'eficiència de metilació de l'As i les puntuacions de MSCA.

ABSTRACT

Introduction: Manganese (Mn) is an essential element that is indispensable for correct development during the foetal stage. Arsenic (As) is toxic to humans, its inorganic form (iAs) being considered the most toxic. The main route of exposure to both compounds in the general population is through diet. Once absorbed, iAs is biotransformed into monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA). Several epidemiological studies have linked prenatal exposure to these metals to adverse effects on neuropsychological development during childhood, although the current evidence is inconclusive. Moreover, most of these studies have been conducted in areas with high environmental exposure to these compounds and evidence in areas with lower levels in the environment, including food and water, is still scarce. The **general aim** of the present thesis is to study the relationship between prenatal exposure to Mn and As and the occurrence of adverse effects on child neuropsychological development in participants from two maternal-infant cohorts, as well as to evaluate the factors associated with these exposures.

Methods: This study is part of the multicentre cohort-based INMA (Infancia y Medio Ambiente [Environment and Childhood]) project. The study population was between 807 and 1179 mother–child pairs from the Gipuzkoa and Valencia cohorts (2004-2008). During the first trimester of pregnancy, serum and urine samples were collected and analysed to determine the concentrations of Mn and total As (TAs) and its metabolites (monomethylarsonic acid [MMA], dimethylarsinic acid [DMA], inorganic As [iAs] and arsenobetaine [AB]), respectively. The children's neuropsychological development was assessed using the Bayley Scales of Infant Development (BSID) at 1 year of age and the McCarthy Scales of Children's Abilities (MSCA) at 4-5 years of age. Sociodemographic, lifestyle and dietary information was collected by questionnaires, together with other maternal biomarkers (serum ferritin and urinary zinc [Zn], among others). The As methylation efficiency was determined through metabolite percentages and using principal component analysis. Multivariate linear regression models were used to evaluate factors associated with exposure variables, as well as to assess the relationship between prenatal exposure to Mn, to As and its metabolites, and As methylation efficiency and neuropsychological test scores. Finally, the linearity of the relations and the possible effect modification related to several variables was explored.

Results: The geometric mean (GM) and 95% confidence interval (95%CI) of serum Mn concentrations during the first trimester of pregnancy was 1.50 µg/L (1.48-1.53). The only dietary factor associated with these concentrations was the consumption of nuts. Working mothers had lower serum Mn concentrations. The GM (95% CI) of TAs, AB, ∑As (sum of DMA, MMA and iAs),

DMA, MMA and iAs concentrations in urine were 35.55 (33.10-38.19), 20.17 (18.34-22.19), 7.74 (7.41-8.09), 6.82 (6.52-7.14), 0.34 (0.32-0.36) and 0.33 (0.31-0.35) µg/g creatinine, respectively. Rice consumption was directly associated with urinary concentrations of all As metabolites except AB. Similarly, fish consumption was positively associated with concentrations of all the metabolites assessed except MMA and iAs. Other factors associated with the concentrations of certain As species were study area, maternal country of origin, parental social class and maternal body mass index (BMI). Regarding As methylation efficiency during pregnancy, the medians (95%CI) of the percentage of As metabolites were 89.7 (89.3-90.2) for %DMA, 5.1 (4.8-5.3) for %MMA and 4.7 (4.5-5.0) for %iAs. Women born in Latin America and those with a higher BMI had better iAs methylation (indicated by higher %DMA and lower %MMA and %iAs). In this multicentre cohort study, no association was found between prenatal Mn exposure and mental development (β [95% CI] = −0.39 [−2.73, 1.95]) or with psychomotor development (β [95% CI] = −0.92 [−3.48, 1.65]) of children at one year of age. Regarding prenatal As exposure, in the present work, a negative relationship was observed between urinary MMA concentrations during the first trimester of pregnancy and scores on the general, verbal, quantitative, memory, executive function and working memory scales of the MSCA at 4-5 years of age (e.g. β [95% CI] = −1.37 [−2.33, −0.41] for the general scale). In addition, %MMA was negatively associated with the memory subscale. Finally, it was observed that children whose mothers had lower concentrations of manganese, zinc and ferritin scored lower on several MSCA scales with decreasing methylation efficiency.

Conclusions: The levels of Mn, As and its metabolites analysed in our population are lower than those observed in most of the studies published to date. Certain dietary, lifestyle and environmental factors influence both the concentrations of Mn and As species and the As methylation efficiency in our population. This study shows no association between prenatal maternal Mn levels and neuropsychological development at one year after birth. However, an inverse association between MMA concentrations and the neuropsychological development of children aged 4-5 years has been observed. In addition, maternal Mn, Zn and ferritin levels modified the association between As methylation efficiency and MSCA scores.

TABLA DE CONTENIDOS

ÍNDICE DE TABLAS Y FIGURAS

Índice de tablas

Índice de figuras

ABREVIATURAS

a	Años
AB	Arsenobetaína
ADN	Ácido desoxirribonucleico
AIC	Criterio de información de Akaike
As	Arsénico
As^{III}	Arsenito
As^{V}	Arsenato
AS3MT	Arsenito metiltransferasa
ATG	Arsénico triglutation
BGV	Valor de referencia biológica
BMDL	Benchmark dose lower level (nivel inferior de dosis de referencia)
BSID	Escalas de Desarrollo Infantil de Bayley
Cd	Cadmio
CI	Cociente intelectual
CFA	Cuestionario semicuantitativo de frecuencia alimentaria
d	Días
DE	Desviación estándar
DMA	Ácido dimetilarsínico
DOHaD	Hipótesis de los orígenes de la salud y enfermedad en el desarrollo
EEUU	Estados Unidos de América
EFSA	European Food Safety Authority (Autoridad Europea de Seguridad Alimentaria)
FAO	Organización de las Naciones Unidas para la Agricultura y la Alimentación
Fe	Hierro
FISABIO	Fundación para la Investigación Sanitaria y Biomédica de la Comunidad Valenciana
GAM	Modelos aditivos generalizados
GSH	Glutatión
GSTO1	Glutatión S-transferasa omega-1
IARC	International Agency for Research on Cancer
iAs	Arsénico inorgánico
ICPMS	Espectrometría de masas de plasma acoplado inductivamente
ICPMS/MS	Espectrómetro de masas en tándem de plasma acoplado inductivamente
IC95%	Intervalo de confianza del 95%
IOM	Institute of Medicine
LDL	Lipoproteína de baja densidad
LOD	Límite de detección

LRT	Test de razón de verosimilitudes
IMC	Índice de masa corporal
INMA	Infancia y Medio Ambiente
m	Meses
MADG	Monometilarsénico diglutatión
METALIA	METALs and chIdren's heAlth
MG	Media geométrica
MMA	Ácido monometilarsónico
MMT	Tricarbonilo metilciclopentadienilo de manganeso
Mn	Manganeso
MSCA	Escalas de Habilidades Infantiles McCarthy
NO_2	Dióxido de nitrógeno
oAs	Compuestos orgánicos de As
OCM	Metabolismo de 1 carbono
OMS	Organización Mundial de la Salud
PC	Componente principal
PCA	Análisis de componentes principales
PCB	Bifenilos policlorados
PFIS	Ayuda Predoctoral para Formación de personal en Investigación en Salud
$PM_{2.5}$	Partículas finas con diámetro inferior a 2,5 micras
PM_1	Partículas finas con diámetro inferior a 1 micra
PNP	Purina nucleósido fosforilasa
ROS	Especies reactivas de oxígeno
SAM	S-adenosil metionina
Se	Selenio
Sg	Semanas de gestación
SNC	Sistema nervioso central
TAs	Arsénico total
UE	Unión Europea
VIF	Factores de inflación de la varianza
Zn	Zinc
∑As	Suma de DMA, MMA e iAs
µg/L	Microgramo por litro

CAPÍTULO I. INTRODUCCIÓN

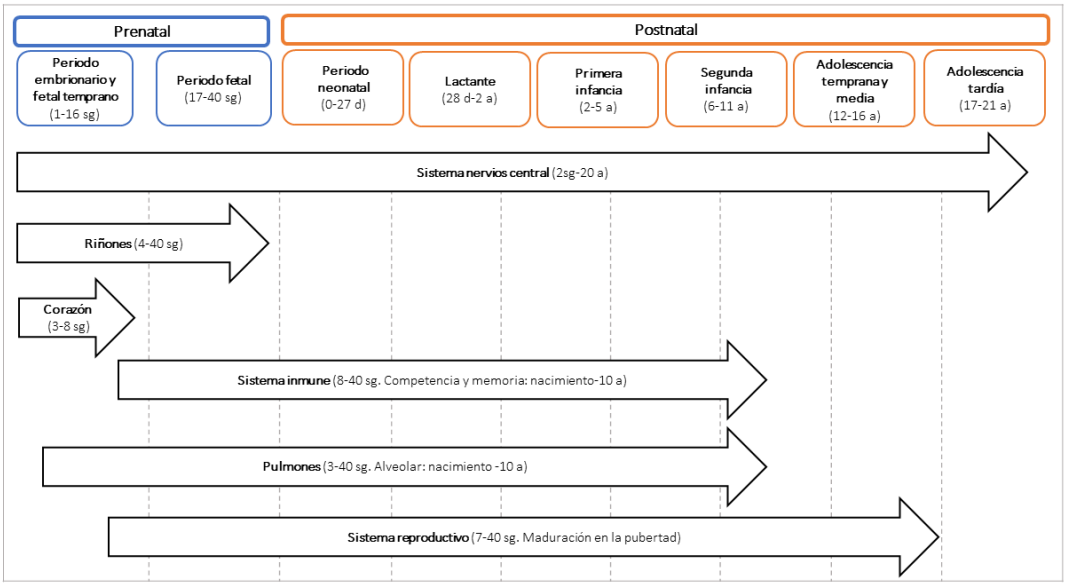
1.1 EXPOSICIÓN A TÓXICOS AMBIENTALES DURANTE ETAPAS VULNERABLES

1.1.1. Vulnerabilidad en etapas tempranas de la vida a la exposición a contaminantes ambientales

1.1.1.1 Vulnerabilidad durante la etapa prenatal

Durante la etapa prenatal la placenta actúa de barrera protegiendo al feto en desarrollo de las sustancias tóxicas a las que está expuesta la madre. Sin embargo, existe evidencia de que esa protección no es completa. Desde las primeras semanas del embarazo, ciertas sustancias son capaces de atravesar la barrera placentaria mediante mecanismos de difusión pasiva y transporte activo, dependiendo de las características del compuesto (peso molecular, ionización, liposolubilidad y capacidad para unirse a proteínas) (Gundacker et al., 2016; Myren et al., 2007).

Junto a lo anterior, durante el desarrollo intraembrionario los mecanismos de detoxificación son todavía inmaduros (Selevan et al., 2000), provocando la acumulación de sustancias tóxicas en los órganos en desarrollo (Barr et al., 2007). Todo esto sucede durante un periodo donde se produce la diferenciación de los tejidos, la organogénesis y el crecimiento fetal. Estos procesos complejos, que ocurren de manera rápida y altamente coordinada, son muy susceptibles a la exposición a sustancias tóxicas o a niveles inadecuados de diversos elementos esenciales. Es lo que se conoce como ventanas de vulnerabilidad (ver **Figura 1**) (Etzel y Landrigan, 2014).



Adaptado de Etzel y Landrigan (2014).

Nota: sg: semanas de gestación; d: días; m: meses; a: años.

1.1.1.2 Vulnerabilidad durante la infancia

Tras el nacimiento, los niños y niñas siguen expuestos a contaminantes ambientales que pueden afectar su desarrollo. Durante este periodo, tanto las fuentes de exposición a sustancias tóxicas, como los efectos producidos por las mismas, son diferentes a la de la población adulta. Esto se debe a ciertas particularidades propias que, además, van cambiando a lo largo de las diferentes etapas, desde el periodo neonatal hasta la adolescencia (Cohen Hubal et al., 2014).

Una de estas características es la mayor exposición a sustancias tóxicas durante la infancia, debido a una mayor ingesta y tasa respiratoria en relación a su peso corporal. Además, durante los primeros meses de vida, los lactantes quedan expuestos a ciertas sustancias a los que ha estado expuesta su madre, a través de la lactancia materna, lo que supone una vía específica durante esta etapa (Rebelo y Caldas, 2016). Tras este periodo, la dieta durante la infancia es sustancialmente diferente a los adultos (por ejemplo, mayor ingesta de agua, zumos, arroz y productos basados en éste y otros cereales). Finalmente, durante este periodo se produce una mayor y diferente exposición a sustancias debido a comportamientos propios de cada una de las etapas de desarrollo (por ejemplo, mayor conducta exploratoria, mayor tiempo de actividad en el suelo y al aire libre, comportamiento mano-objeto/boca) (Cohen Hubal et al., 2014; Etzel y Landrigan, 2014; U.S. Environmental Protection Agency, 2008; World Health Organization, 2005).

Además de una mayor exposición, durante la infancia los sistemas y mecanismos de detoxificación, metabolismo y excreción siguen siendo inmaduros, especialmente en los primeros

doce meses tras el nacimiento. Así mismo, en el periodo postnatal los órganos siguen en proceso de desarrollo, algunos como el sistema nervioso hasta la adolescencia. Esto lleva a que estos órganos y estructuras en crecimiento sigan siendo más vulnerables a los efectos tóxicos de ciertas sustancias (ver **Figura 1**) (Etzel y Landrigan, 2014; U.S. Environmental Protection Agency, 2008; World Health Organization, 2005).

1.1.2. Efectos de la exposición prenatal. Hipótesis de los orígenes de la salud y enfermedad en el desarrollo (DOHaD)

La hipótesis de los orígenes de la salud y enfermedad en el desarrollo (DOHaD, por sus siglas en inglés) postula que el ambiente en el que se encuentra un individuo durante su desarrollo temprano puede tener un impacto sobre la salud a lo largo de su vida (Barker, 2007). Esta teoría fue propuesta originalmente por Barker en los años 80. En sus estudios se observó que la malnutrición durante el embarazo y la infancia podía llevar a cambios estructurales, fisiológicos y metabólicos permanentes, lo que podría favorecer la aparición de enfermedades cardiovasculares durante la edad adulta (Barker, 2007).

La hipótesis DOHaD ha seguido desarrollándose y ampliándose durante las últimas décadas, encontrando nuevas evidencias sobre la influencia del ambiente durante el desarrollo, no solo del estado nutricional materno, si no también otros factores, como el tabaquismo, el estrés y la exposición a tóxicos ambientales. Respecto a este último, la literatura ha aumentado considerablemente, poniendo el foco particularmente en el impacto que puede tener la exposición a tóxicos, como los bifenilos policlorados (PCB), la contaminación atmosférica y ciertos metales (por ejemplo, mercurio, plomo y arsénico) sobre ciertos aspectos de la salud infantil, entre ellos en el desarrollo neuropsicológico (Abdul-Hussein et al., 2020; Heindel et al., 2017; Zheng et al., 2016).

Aunque el mecanismo aún no está claro, la evidencia sugiere que el ambiente durante el desarrollo podría tener una influencia sobre la regulación epigenética del individuo. Esto es, mediante diferentes mecanismos se producen alteraciones en la expresión de ciertos genes sin modificar la secuencia del ADN, llamadas modificaciones epigenéticas, como la metilación del ADN o la modificación de las histonas y los ARN no codificantes, entre otras. Estos cambios podrían influir en la expresión genética de manera permanente, lo que puede llevar a una mayor susceptibilidad a desarrollar enfermedades a lo largo de la vida (Barouki et al., 2018; Heindel et al., 2015; Vineis et al., 2017). Otros mecanismos de acción propuestos para el desarrollo de enfermedades son el estrés oxidativo y la inflamación crónica inducida por la exposición a tóxicos durante las etapas vulnerables, interferencias en los sistemas dopaminérgicos, colinérgicos y

glutamatérgico, disrupción endocrina, disfunción mitocondrial, alteración metabólica, alteraciones estructurales de los órganos en desarrollo así como deterioro de la plasticidad neuronal (Almeida et al., 2019; Grilo et al., 2021; Karmaus et al., 2019; Schug et al., 2013).

1.1.3. Vulnerabilidad del sistema nervioso infantil frente a la exposición a tóxicos ambientales

Como se ha comentado anteriormente, durante el periodo embriofetal se produce la diferenciación de tejidos y la organogénesis. El desarrollo del sistema nervioso central (SNC) tiene ciertas particularidades que lo diferencia del resto de órganos. La más importante es que el proceso de desarrollo es largo, ya que empieza muy temprano en la etapa prenatal (alrededor de la segunda semana de gestación), y se extiende durante los periodos pre y postnatal hasta la adolescencia (Chang et al., 2015; Stiles y Jernigan, 2010). Este proceso complejo y altamente organizado supone un periodo crítico de vulnerabilidad a la exposición, tanto a sustancias tóxicas, como a niveles inadecuados de elementos necesarios para el desarrollo de este sistema, como el hierro o el manganeso.

Durante esta etapa, la barrera hematoencefálica continúa en desarrollo, por lo que muchos compuestos, por ejemplo metales tales como el arsénico, el manganeso, el plomo o el metilmercurio, son capaces de atravesarla (Lucchini, Aschner, Bellinger, et al., 2015). En el SNC inmaduro un gran número de procesos, mayor que en el adulto, son llevados a cabo a través de neurotransmisores, cuya función puede ser interrumpida debido a la exposición de determinados tóxicos. De la misma manera, el cerebro en desarrollo contiene un número menor de enzimas antioxidantes comparadas con el adulto, lo que lo hace más vulnerable al estrés oxidativo provocado por ciertos compuestos neurotóxicos (Lucchini, Aschner, Bellinger, et al., 2015; Rock y Patisaul, 2018). Además, se ha observado que las neuronas inmaduras y las células neuronales precursoras, fundamentales durante el proceso de neurogénesis, son altamente vulnerables a la exposición a ciertos tóxicos (Abbott y Nigussie, 2021). Desde hace décadas, estudios experimentales y epidemiológicos han puesto de manifiesto como la exposición prenatal a ciertos metales puede tener efectos adversos en el desarrollo neuropsicológico infantil a concentraciones inocuas para los adultos (Grandjean y Landrigan, 2006, 2014).

Tras el nacimiento y durante los primeros años de vida, otra de las fuentes de exposición a tóxicos ambientales es la lactancia materna. El análisis de esta matriz ha evidenciado la presencia de diferentes metales, como mercurio, arsénico, cadmio o plomo, así como otros tóxicos, como dioxinas, bisfenoles, parabenos o ftalatos (Rebelo y Caldas, 2016; Yusà et al., 2020).

Algunos de los posibles mecanismos propuestos en el desarrollo de efectos adversos en el neurodesarrollo debido a la exposición pre y postnatal a sustancias tóxicas son los siguientes (Oulhote y Bellinger, 2019; Rock y Patisaul, 2018):

- El estrés oxidativo, que puede conducir a la muerte de células neuronales. Como se ha comentado anteriormente, el cerebro en desarrollo es especialmente vulnerable a los radicales libres formados a través de este mecanismo.
- La alteración de los sistemas de neurotransmisores, como la dopamina o la serotonina, lo que puede llevar a efectos en la función cerebral.
- Disrupción neuroendocrina, que puede afectar a la diferenciación celular cerebral y la sinaptogénesis
- Cambios epigenéticos, como modificaciones en la metilación del ADN, que podrían afectar la expresión de genes relacionados con funciones cerebrales.
- Disrupción del sistema inmune, cuya activación en periodos críticos, como la etapa prenatal, puede llevar a cambios en la estructura y función del cerebro en desarrollo.

1.1.4. Impacto en salud debido a la exposición a neurotóxicos durante etapas vulnerables

La exposición a sustancias neurotóxicas podría ser, en parte, causa de la elevada prevalencia de los trastornos del desarrollo neuropsicológico en la infancia. En el 2016, se estimó que el número de casos a nivel global de niños y niñas menores a 5 años que presentaron discapacidad intelectual fue de más de 12,5 millones, lo que representa una prevalencia de 20 casos por cada 1000 habitantes. La prevalencia de este tipo de trastornos es más alta en países en desarrollo; sin embargo, este es un problema global. En España, durante el mismo año, la prevalencia se situó en 15 casos por cada 1000 habitantes (Olusanya et al., 2018).

Además, es posible que la prevalencia de trastornos del desarrollo neuropsicológico esté infraestimada, ya que, en ocasiones se utilizan instrumentos de medida que se centran en signos de discapacidad y pueden no ser capaces de detectar problemas leves o moderados relacionados con el deterioro cognitivo o problemas de comportamiento (World Health Organization, 2012). Este hecho implicaría que el impacto de la exposición a sustancias neurotóxicas en el desarrollo infantil pueda ser aún mayor del actualmente considerado. Por ejemplo, en el estudio de Zablotsky (2017), se observó que la prevalencia de discapacidad del desarrollo en EE. UU., entre 2014 y 2016, (donde se incluyeron, no solo casos de desorden del espectro autista y discapacidad intelectual, sino cualquier otro retraso en el desarrollo diagnosticado) fue del 7% .

Aun siendo complejo, diversos estudios han intentado estimar la contribución de los tóxicos ambientales como factores de riesgo para el neurodesarrollo durante la infancia. Una revisión estimó el impacto de ciertos factores de riesgo ambientales (exposición a plomo, metilmercurio y plaguicidas organofosforados) en el cociente intelectual (CI) en niños y niñas menores de cinco años en EE. UU. Desde esta perspectiva poblacional, se observó que estos tres riesgos ambientales suponían una contribución importante a la pérdida de puntuación de CI, no solo por la magnitud del efecto de estos químicos sobre el neurodesarrollo, sino por la amplia prevalencia de población infantil expuesta a estos riesgos (Bellinger, 2012). Por otro lado, un estudio sobre la estimación de carga de enfermedad debida a factores ambientales en población infantil de 28 países europeos estimó que, del total de exposiciones ambientales evaluadas, la exposición a plomo contribuyó en un 3% a los años de vida perdidos ajustados por discapacidad (Rojas-Rueda et al., 2019).

Como se observa, la exposición a compuestos neurotóxicos durante periodos vulnerables podría tener un impacto directo sobre la salud infantil, e incluso causar efectos adversos a lo largo de la vida. Continuar estudiando cómo afectan estas sustancias en diferentes áreas geográficas, con distintos niveles de exposición, proporcionará información valiosa mediante la identificación de poblaciones más susceptibles a los posibles efectos derivados de la exposición a estos compuestos, así como la detección de aquellas en alto riesgo de exposición. Este conocimiento podrá ayudar a la implementación de medidas que promuevan un cambio hacia un entorno más saludable en las etapas vulnerables, lo que proporcionará beneficios en salud a corto y largo plazo.

1.2. MANGANESO

El manganeso (Mn) es un metal de transición, que se encuentra en el ambiente de forma natural, siendo el quinto metal más abundante de la corteza terrestre. Para los humanos, el Mn actúa como elemento esencial, es decir, participa en numerosos procesos metabólicos, y su ausencia o deficiencia podría causar efectos negativos funcionales y estructurales (Aggett et al., 2015).

1.2.1. Fuentes de emisión

El Mn se encuentra de manera natural en el ambiente, como componente de más de cien minerales (Agency for Toxic Substances and Disease Registry, 2012). No obstante, son las fuentes antropogénicas las que contribuyen sustancialmente a aumentar los niveles de Mn en el ambiente. Las principales fuentes de emisión de Mn incluyen la minería, la producción de aleación de ferromanganeso y la fabricación de hierro y acero (Agency for Toxic Substances and Disease Registry, 2012; Lucchini, Aschner, Kim, et al., 2015).

En el aire, en áreas rurales y urbanas, este compuesto se encuentra en concentraciones bajas como componente del material particulado (PM), aumentando considerablemente los niveles en áreas cercanas a industrias de ferro y silicomanganeso debido a las emisiones producidas por este tipo de factorías (Lucchini, Aschner, Kim, et al., 2015; World Health Organization, 2000a). El uso del aditivo de la gasolina tricarbonilo metilciclopentadienilo de manganeso (MMT) también contribuye a aumentar los niveles de este compuesto en la atmósfera, aunque su uso está limitado en la Unión Europea (UE) (European Commission, 2009; Lucchini, Aschner, Kim, et al., 2015). De la misma manera, el uso de ciertos productos agrícolas, especialmente el fungicida mancoceb, aumenta los niveles en el ambiente, aunque el uso de este producto ha sido prohibido en la UE recientemente (European Commission, 2020a).

El Mn también está presente de manera natural tanto en el suelo, como en el agua. No obstante, al igual que en al aire, las fuentes antropogénicas contribuyen en gran medida a la contaminación de estas fuentes. El Mn presente en el suelo puede migrar al agua, especialmente las formas solubles de este compuesto (Lucchini, Aschner, Kim, et al., 2015; World Health Organization, 2011). En el agua, el Mn está presente principalmente en aguas subterráneas, debido a las condiciones anaeróbicas de las mismas (World Health Organization, 2017).

1.2.2. Vías de exposición

1.2.2.1 Exposición en población general

Exposición dietética

En población general, la principal vía de exposición a este elemento es a través de la dieta. El Mn es un componente natural de la mayoría de los alimentos. Sin embargo, se observa una variación considerable en los niveles según el tipo de producto. En ciertos alimentos de origen vegetal, como los frutos secos y semillas, chocolate, té, frutas y los cereales, se han observado niveles altos de Mn (Agence nationale de sécurité sanitaire de l'alimentation de l'environnement et du travail, 2011; Filippini et al., 2017; Perelló et al., 2015; Rose et al., 2010; U.S. Food and Drug Administration, 2017). Los cereales, así como los productos basados en ellos, constituyen el grupo de alimentos que contribuyen en mayor medida a la exposición de Mn a través de la dieta, debido tanto a presentar niveles relativamente altos de este elemento, como a su alta frecuencia de consumo (Agence nationale de sécurité sanitaire de l'alimentation de l'environnement et du travail, 2011; EFSA Panel on Dietetic Products Nutrition and Allergies, 2013). Por otra parte, se ha observado que ciertos hábitos alimenticios pueden aumentar la ingesta de Mn, por ejemplo las dietas vegetarianas (Freeland-Graves et al., 2016).

Otra ruta de exposición a Mn es a través del consumo de agua potable, especialmente en zonas donde los niveles en este medio son altos, como en ciertas áreas de Bangladesh, Japón o Australia. Algunos compuestos de Mn son fácilmente solubles, por lo que la exposición puede ocurrir por la ingestión de agua potable contaminada (Lucchini, Aschner, Kim, et al., 2015; World Health Organization, 2011).

En lactantes, otra vía de exposición a este compuesto es a través de la leche materna y las fórmulas infantiles. Las concentraciones de Mn en leche materna varían entre 3 y 30 µg/L (EFSA Panel on Dietetic Products Nutrition and Allergies, 2013). En leche de fórmula, el contenido en Mn en comparación es significativamente superior que en leche materna (32-367 µg/L) (Ljung et al., 2011). Un reciente estudio ha estimado que la ingesta mínima de Mn a través de estos productos es, al menos, 28 veces superior que la ingesta media a través de leche materna (Mitchell et al., 2020).

Exposición inhalada y dérmica

Como se ha comentado anteriormente, las concentraciones atmosféricas de Mn varían considerablemente dependiendo de la proximidad a fuentes de emisión de este elemento. Así, en zonas cercanas a fundiciones y a ciertas industrias, las concentraciones de Mn aumentan significativamente (Agency for Toxic Substances and Disease Registry, 2012; World Health Organization, 2000a). Se ha propuesto que otra fuente de exposición de Mn inhalado es a través del consumo de tabaco, aunque los niveles de Mn en el tabaco no parecen ser altos (Agency for Toxic Substances and Disease Registry, 2012). Respecto a la exposición dérmica, en población general esta vía se considera muy rara debido a que este compuesto no penetra con facilidad a través de la piel (Agency for Toxic Substances and Disease Registry, 2012).

Exposición prenatal

El Mn es capaz de atravesar la barrera placentaria a través de mecanismos activos de trasporte, con el fin de satisfacer la demanda fetal de este elemento (Nandakumaran et al., 2016). Durante la gestación se ha observado un aumento de las concentraciones de Mn en la sangre materna (Spencer, 1999; Takser et al., 2004). Se cree que esta variación en los niveles se debe principalmente a cambios fisiológicos producidos durante este periodo, como por ejemplo una mayor absorción intestinal de Mn debido a la deficiencia fisiológica del hierro, elemento con el cual comparte mecanismos de transporte (Agency for Toxic Substances and Disease Registry, 2012; Kim, 2018).

1.2.2.2 Exposición ocupacional

Exposición inhalada y dérmica

En el ámbito ocupacional, la exposición a este compuesto se produce a través de la inhalación de vapores de Mn o polvos que contienen este metal. Los sectores ocupacionales que presentan mayor exposición son la minería, la producción y fundición de metales, la producción y uso de productos agrícolas y la fabricación de baterías (European Commission Employment Social Affairs and Inclusion, 2011). La exposición a Mn a través de la inhalación parece ser más tóxica, debido a que el Mn se transporta directamente al cerebro a través del tracto olfativo antes de ser metabolizado en el hígado (Agency for Toxic Substances and Disease Registry, 2012; Lucchini, Aschner, Kim, et al., 2015). Respecto a la exposición dérmica a Mn, como se ha comentado anteriormente, esta es muy rara. Únicamente se ha informado de exposiciones ocupaciones debido a la manipulación de MMT (Lucchini, Aschner, Kim, et al., 2015).

1.2.3. Toxicocinética del manganeso

El Mn ingerido por la dieta es absorbido a través del tracto gastrointestinal mediante transportadores no específicos que regulan la absorción de otros metales, como el hierro (Fe) (ver **Figura 2**). De hecho, se ha observado que la deficiencia de Fe aumenta la absorción de Mn en el intestino, debido a la competencia de ambos metales por los transportadores (EFSA Panel on Dietetic Products Nutrition and Allergies, 2013; Finley, 1999; Kim y Park, 2014). Una vez es absorbido, el Mn se distribuye rápidamente a los órganos a través de la sangre, principalmente unido a los eritrocitos. Los niveles de este elemento en la sangre se mantienen dentro de unos límites fisiológicos estables, debido a la regulación de la absorción gastrointestinal y la excreción hepatobiliar. El hígado es el principal órgano regulador de la homeostasis de este elemento en el cuerpo, eliminando el exceso de Mn mediante la conjugación con la bilis y la excreción a través de las heces (Agency for Toxic Substances and Disease Registry, 2012; Chen et al., 2018).

No obstante, este mecanismo homeostático puede verse afectado por diferentes condiciones. Como se ha comentado anteriormente, se ha observado una influencia del hierro en la absorción de Mn (Finley, 1999; Kim y Park, 2014). Así mismo, la exposición a niveles muy altos a este elemento, especialmente en ambientes ocupacionales, puede sobrepasar el mecanismo regulador fisiológico y provocar una acumulación de niveles elevados en el cuerpo. Finalmente, las enfermedades hepáticas pueden llevar a una mayor absorción de este elemento (Agency for Toxic Substances and Disease Registry, 2012).

Debido a sus numerosas funciones dentro del organismo, este elemento se encuentra en todos los tejidos a niveles estables. No obstante, en ciertos órganos y tejidos se observa una mayor acumulación de Mn, como en el hígado, el páncreas, los huesos, el colon, los riñones y, especialmente, en zonas específicas del cerebro (*globus pallidus*) (Agency for Toxic Substances and Disease Registry, 2012; Tuschl et al., 2013) .

Respecto al Mn inhalado, este es absorbido en los pulmones, entrando en la circulación sistémica. No obstante, a través de la inhalación también puede ser transportado al bulbo olfatorio desde donde pasa al cerebro mediante ciertos transportadores, evitando la metabolización y regulación por parte del hígado (Chen et al., 2018)

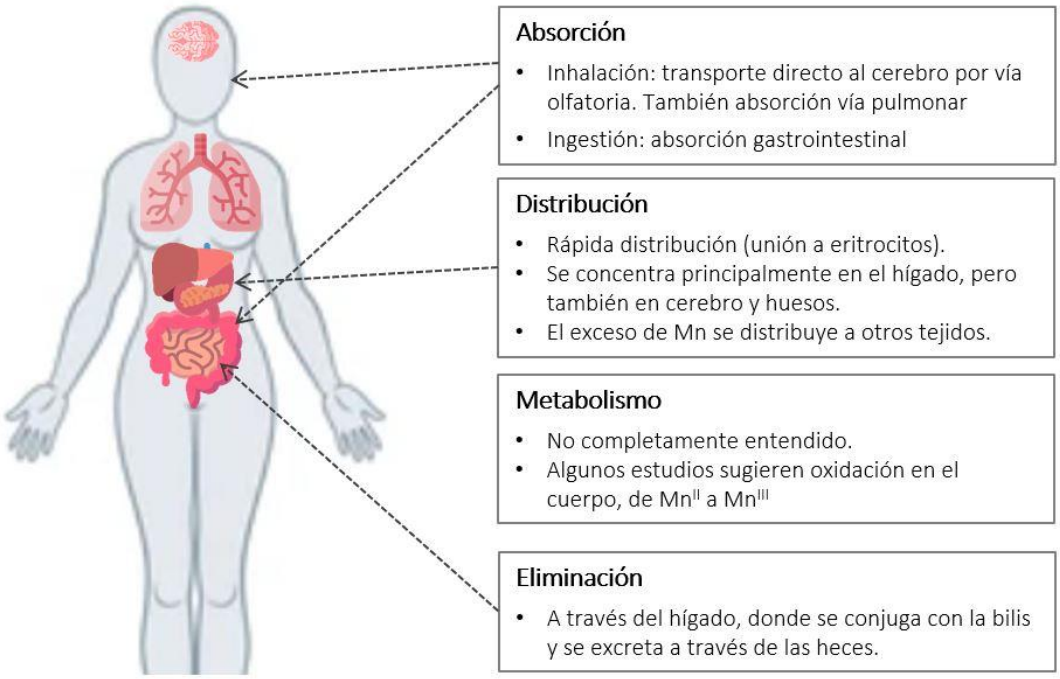


Figura 2. Toxicocinética del manganeso.

Nota: Elaboración propia. Imágenes: shutterstock y Flaticon.

1.2.4. Funciones fisiológicas del manganeso

El Mn es un elemento esencial para el ser humano. Este compuesto es necesario para el mantenimiento de la función cerebral y nerviosa, ya que actúa como un importante cofactor en enzimas involucradas en la síntesis y metabolismo de neurotransmisores. Además, forma parte de numerosos procesos metabólicos, como en el metabolismo de grasas y carbohidratos, así como la formación de tejido conectivo y esquelético (Agency for Toxic Substances and Disease Registry, 2012; Santamaria y Sulsky, 2010). Basándose en datos de estudios de dieta total, la European Food Safety Authority (EFSA) (2013) observó una ingesta media de entre 2 a 6 mg/ día de Mn en la UE, por lo que estableció una ingesta adecuada diaria de Mn de 3mg para evitar la deficiencia de este elemento.

1.2.5. Efectos en salud por exposición a manganeso adultos

Aunque el déficit de Mn no se ha descrito en humanos, la exposición a niveles altos de este elemento sí que ha mostrado tener efectos en salud, principalmente efectos neurotóxicos.

1.2.5.1 Efectos neurotóxicos

El principal efecto en salud debido a altas exposiciones de Mn es un síndrome parkinsoniano denominado manganismo. Este trastorno neurológico incluye síntomas motores, como temblores, desórdenes de la marcha, y espasmos en músculos faciales, así como síntomas psiquiátricos, como alucinaciones. Este trastorno está causado por la acumulación de Mn en ciertas zonas del cerebro (Miah et al., 2020). Existe evidencia concluyente de que la inhalación de altos niveles de Mn puede llevar a desarrollar dicho síndrome, sobre todo en ambientes ocupacionales y en poblaciones cercanas a ciertas industrias. No obstante, también se han descrito casos tras la exposición oral a altos niveles de este compuesto, especialmente a través del agua (Agency for Toxic Substances and Disease Registry, 2012; EFSA Panel on Dietetic Products Nutrition and Allergies, 2013). Además, en un reciente metaanálisis se observó una correlación negativa significativa entre la exposición a Mn inhalado en adultos y funciones cognitivas y motoras, aunque la magnitud del efecto fue pequeña (Ruiz-Azcona et al., 2021).

1.2.5.2 Otros efectos en salud

Se han observado efectos respiratorios debido a exposiciones de Mn por vía inhalatoria, como inflamación pulmonar y disminución de la función pulmonar, este último en fumadores (Agency for Toxic Substances and Disease Registry, 2012; Wang et al., 2015). Además, existe evidencia, todavía no concluyente, de que la exposición a este elemento parece tener efectos sobre la función cardiovascular, produciendo alteraciones electrocardiográficas e hipotensión (Agency for Toxic Substances and Disease Registry, 2012; Jiang y Zheng, 2005; O´Neal y Zheng, 2015).

1.2.6. Efectos en salud por exposición a manganeso durante etapas vulnerables

1.2.6.1 Efectos en el desarrollo neuropsicológico por exposición temprana a Mn

La evidencia epidemiológica sobre los efectos de la exposición prenatal a Mn en el desarrollo neuropsicológico durante la infancia es escasa. En el estudio de Takser et.al (2003) se encontró una asociación inversa entre los niveles de Mn analizados en sangre de cordón umbilical (media geométrica 38,5 µg/L) y las puntuaciones en habilidades manuales, atención y memoria no verbal a los tres años de edad. Sin embargo, no se encontró asociación entre los niveles de Mn y el desarrollo cognitivo general a los seis años. En otro estudio llevado a cabo en Taiwán, se observaron peores resultados en la evaluación del desarrollo cognitivo y del lenguaje en los niños y niñas que presentaron niveles de Mn en sangre de cordón por encima de 59,59 µg/L (Lin et al., 2013). En el estudio de Claus-Henn (2017), realizado en EEUU, se observó una asociación inversa

significativa entre las concentraciones de Mn en sangre materna medidos en el parto (mediana = 24,0 µg/L) y el desarrollo mental y psicomotor a los dos años de edad. Resultados similares se observaron en un estudio mexicano (Muñoz-Rocha et al., 2018), donde los niveles de Mn en sangre materna (media= 27,7 µg/L) se asociaron de manera negativa con la evaluación de la función cognitiva, motora y del lenguaje a los dos años de edad. No obstante, en el mismo estudio se observó una relación no lineal cuando el biomarcador de exposición fue evaluado en sangre de cordón (media= 50,1 µg/L). Esta forma de relación de U invertida, característica de los elementos esenciales, también se encontró en una cohorte coreana para el desarrollo mental y psicomotor a los seis meses de edad en el que se observó un cambio en la asociación de positiva a negativa para niveles de Mn en sangre materna de alrededor de 24-28 µg/L (Chung et al., 2015). Por su parte, diversos estudios no han observado ninguna relación entre los niveles de Mn prenatal y efectos en el neurodesarrollo durante la infancia (Kupsco et al., 2020; Mora et al., 2018). Finalmente, una revisión sistemática de estudios observacionales concluyó que la evidencia actual sobre los efectos de la exposición a Mn en estadios tempranos (gestación y primeros años de vida) son todavía no concluyentes para establecer una relación causa-efecto (Leonhard et al., 2019)

De la misma manera que en la exposición prenatal, la exposición postnatal a Mn en los primeros años de vida, la infancia y la adolescencia ha sido relativamente poco estudiada. Para estimar la exposición a Mn, diversos estudios epidemiológicos han analizado tanto la exposición a través del agua como las concentraciones de este mineral en diversos biomarcadores (pelo, sangre, suero, dentina). Los resultados sugieren que exposiciones altas a Mn se relacionan negativamente con el desarrollo cognitivo, la función ejecutiva, la función verbal y la memoria (Bjorklund et al., 2017; Lucchini et al., 2017). Alguno de estos estudios han encontrado una relación no lineal, de U invertida (Leonhard et al., 2019). No obstante, aunque existe cierta evidencia sobre la exposición postnatal y su impacto en el neurodesarrollo infantil, el tipo de estudio utilizado (transversal) y la variabilidad en la forma de medir tanto la exposición como el resultado hace que no se pueda establecer una relación causal sólida (Leonhard et al., 2019).

1.2.6.2 Otros efectos en salud por exposición temprana a Mn

Existe evidencia sobre la relación entre la exposición prenatal a Mn y las medidas antropométricas al nacimiento. Diversos estudios han encontrado una relación inversa, aunque no lineal, entre las concentraciones de Mn prenatal y el peso al nacer (Eum et al., 2014; Hu et al., 2018; Wang et al., 2021; Yamamoto et al., 2019). Otro estudio mostró que niveles más bajos de Mn medidos en el tercer trimestre del embarazo se asociaron con un menor peso al nacer, aunque no se observó la misma asociación a concentraciones altas (Ashley-Martin et al., 2018). Recientemente, un estudio ha sugerido que la relación entre la exposición prenatal a Mn y los efectos negativos en

el neurodesarrollo puede estar mediada, precisamente, por la relación entre esta misma exposición y la talla al nacimiento (Lee et al., 2018). En contraste, un estudio transversal ha observado una relación positiva entre la exposición postnatal a Mn y el índice de masa corporal (IMC) y la circunferencia de la cintura en niños y niñas entre 6 y 11 años de edad (Signes-Pastor et al., 2021).

Finalmente, un reciente estudio ha encontrado una relación no linear entre las concentraciones maternas perinatales de Mn y el riesgo de que su descendencia presente presión arterial elevada a los 6-12 años de edad (Wang et al., 2021).

1.2.7. Niveles de referencia e ingesta recomendada de Mn

Como se ha comentado anteriormente, el Mn es un elemento esencial que interviene en numerosas funciones y procesos en el organismo. Diversos mecanismos homeostáticos contribuyen al mantenimiento de los niveles de Mn dentro de los límites fisiológicos (Lucchini, Aschner, Kim, et al., 2015). Los rangos normales de este elemento se encuentran entre 4-15 µg/L en sangre, 1-8 µg/L en orina y 0,4-0,8 µg/L en suero (Agency for Toxic Substances and Disease Registry, 2012).

Aunque la deficiencia de Mn en humanos no se ha descrito, tanto el Institute of Medicine (IOM) en el año 2006 (Institute of Medicine, 2006),como la EFSA en el año 2013 (EFSA Panel on Dietetic Products Nutrition and Allergies, 2013) establecieron unos valores de ingesta adecuada diaria de Mn de en adultos, incluyendo el embarazo y la lactancia (3mg en el caso de la EFSA y 1,8 y 2,3 mg en el caso del IOM). No obstante, la falta de evidencia impidió a la EFSA establecer otros valores nutricionales de referencia, como por ejemplo el nivel superior de ingesta tolerable. Sin embargo, debido a los posibles efectos negativos en salud que se han observado en exposiciones altas a este elemento, en el 2006, el IOM sí estableció una ingesta diaria superior tolerable a través de la dieta en adultos, mujeres embarazadas y mujeres lactantes de 11 mg (Institute of Medicine, 2006) (Tabla 1).

En etapas vulnerables, como la infancia y la adolescencia, la ingesta diaria recomendada y el límite superior tolerable varía según la edad, aunque en cualquier caso es menor que en los adultos (EFSA Panel on Dietetic Products Nutrition and Allergies, 2013; Institute of Medicine, 2006). De la misma manera, la Comisión Europea estableció en el 2015 el contenido mínimo y máximos de Mn para preparados para lactantes y preparados de continuación (1-100 µg/100 kcal) (European Commission, 2015a).

Respecto a los niveles de Mn en agua, la Organización Mundial de la Salud estableció una concentración máxima de 400 µg/L (World Health Organization, 2017). No obstante, en la UE el límite establecido es de 50 µg/L en agua de consumo, debido principalmente a cuestiones estéticas y de aceptabilidad del consumidor, ya que, incluso a niveles bajos (20µg/L), el Mn se deposita en las tuberías y puede enturbiar el agua (European Commission, 2020b).

Por último, respecto a la exposición de Mn por inhalación, la Comisión Europea estableció en el año 2011 un valor límite indicativo de exposición laboral 0,20mg/m^3 (European Commission Employment Social Affairs and Inclusion, 2011).

Ingesta diaria adecuada (mg) [1]						
0-6 m	**7-11 m**	**1-3 a**	**4-6 a**	**7-10 a**	**11-18 a**	**Adultos, mujeres embarazadas y lactancia**
ND	0,02—0,5	1	1,5	2	3	3
Ingesta diaria adecuada (mg) [2]						
0-6 m	**7-11 m**	**1-3 a**	**4-8 a**	**9-13 a**	**14-18 a**	**Adultos, mujeres embarazadas y lactancia**
0,003	0,6	1,2	1,5	1,6—1,9*	1,6—2,2 *	1,8—2,3*
Ingesta diaria superior tolerable (mg) [2]						
0-6 m	**7-12 m**	**1-3 a**	**4-8 a**	**9-13 a**	**14-18 a**	**Adultos, mujeres embarazadas y lactancia**
ND	ND	2	3	6	9	11
Valores Guía máximo de Mn en agua						
				400 µg/L [3]	50 µg/L[4]	
Niveles mínimos y máximos establecidos para preparados para lactantes y preparados de continuación[5]						
				1—100 µg/100 kcal		
Valor límite indicativo de exposición laboral de Mn inhalable[6]						
				0,20mg/m^3		

Tabla 1. Niveles de referencia e ingesta recomendada de manganeso según poblaciones.

Nota: a: años; m: meses. ND: no determinado

[1]EFSA Panel on Dietetic Products Nutrition and Allergies 2013; [2]Institute of Medicine 2006; [3]OMS, 2017; [4] Comisión Europea, 2015; [5]Comisión Europea, 2020; [6]Comisión Europea, 2011.

*Valores según mujer u hombre, respectivamente.

1.3. ARSÉNICO

El arsénico (As) es un metaloide, el cual comparte propiedades tanto de los metales como de los no metales (Agency for Toxic Substances and Disease Registry, 2007). Desde el punto de vista toxicológico, el As puede encontrarse tanto en formas inorgánicas (iAs) como orgánicas. Estas formas, a su vez, se pueden encontrar en distintos estados de oxidación (−3, 0, +3, y +5) (World Health Organization, 2001).

La especiación del arsénico consiste en la identificación y cuantificación de las formas químicas individuales del As, tanto en el ambiente como en sistemas o matrices biológicas. Existen un gran número de compuestos de As. Las formas inorgánicas arsenito (As^{III}) y arsenato (As^{V}) se pueden combinar con grupos metilo ($-CH_3$) formando así las especies metiladas. A continuación, se realiza una clasificación de los compuestos más significativos para el objeto de esta tesis (**Figura 3**).

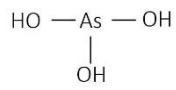
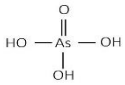
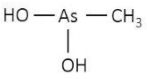
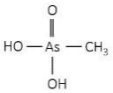
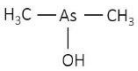
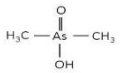
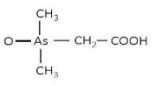
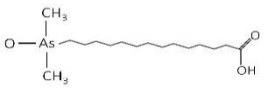
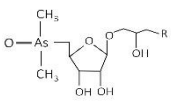
Compuestos inorgánicos		
Arsenito (As^{III}) 	**Arsenato** (As^{V}) 	**As inorgánico** (iAs) Suma de As^{III} y As^{V}
Compuestos orgánicos		
Formas metiladas		
Metilarsenito (MMA^{III}) 	**Metilarsenato** (MMA^{V}) 	**Ácido monometilarsónico** (MMA) Suma de MMA^{III} y MMA^{V}
Dimetilarsenito (DMA^{III}) 	**Dimetilarsenato** (DMA^{III}) 	**Ácido dimetilarsínico** (DMA) Suma de DMA^{III} y DMA^{V}
Compuestos orgánicos complejos		
Arsenobetaína 	**Arsenolípidos** 	**Arsenoazúcares** 

Figura 3. Clasificación de especies de arsénico: nombre (acrónimo) y estructura química.

Nota: elaboración propia.

1.3.1. Fuentes de emisión

El As se encuentra de manera natural en la corteza terrestre y es emitido al medio ambiente debido al desgaste de sedimentos, las emisiones volcánicas, la actividad geotermal y los incendios forestales (Flora, 2015). No obstante, son ciertas actividades humanas las que contribuyen a aumentar de manera significativa la presencia de As en el ambiente (Agency for Toxic Substances and Disease Registry, 2007). Se ha estimado que las emisiones antropogénicas de As son tres veces mayores que las emisiones naturales (World Health Organization, 2000b). Entre estas actividades se encuentran algunos procesos industriales, como la fundición de metales no ferrosos, la combustión de combustibles fósiles, y la minería (European Food Safety Authority, 2009; International Agency for Research on Cancer, 2012). Respecto a esta última actividad, se ha estimado que en Europa, la minería contribuye a la presencia de este tóxico en el ambiente en la misma proporción que los sedimentos producidos por la erosión (Shaji et al., 2021). Además, el As ha sido ampliamente usado en aleaciones de plomo, como conservante de madera, así como en la agricultura, mediante el uso de plaguicidas que contienen este compuesto (Agency for Toxic Substances and Disease Registry, 2007; Flora, 2015). No obstante, en la UE, el uso de este tipo de productos agrícolas no está permitido desde el año 2006 (European Commission, 2006; European Food Safety Authority, 2009) .

El As se transporta principalmente en el medio ambiente a través del agua. El metal presente en las rocas y sedimentos es liberado en el agua subterránea mediante varios procesos. En los sistemas acuáticos, este compuesto se encuentra mayoritariamente en formas inorgánicas, siendo predominante el As^{V} en ambientes con presencia de oxígeno, y el As^{III} en condiciones reductoras (como en aguas profundas) (Flora, 2015). En sedimentos marinos, además, se han encontrado especies metiladas (MMA y DMA), debido a la reducción y metilación del As^{V} por microbios, algas y fitoplancton. Los niveles de As en el agua dependen de diversos factores, como el tipo de sedimento, la concentración de oxígeno o el grado de actividad biológica (European Food Safety Authority, 2009; Flora, 2015; International Agency for Research on Cancer, 2012). En zonas cercanas a áreas industriales o con actividad minera, las concentraciones de As en el agua son más elevadas (Huang et al., 2015).

Por otra parte, la mayor parte del As atmosférico proviene de fuentes antropogénicas, principalmente debido a la quema de combustibles fósiles, incineración de basuras y fundición de metales no ferrosos (Agency for Toxic Substances and Disease Registry, 2007). En este medio, las formas más frecuentes son las inorgánicas pentavalentes, las cuales suelen acumularse en las partículas de menor tamaño (partículas finas con diámetro inferior a 2,5 y 1 micra [$PM_{2.5}$ y PM_{1}, respectivamente]) (Lewis et al., 2012; Nocoń et al., 2020). El As presente en la atmósfera

contribuye, así mismo, a aumentar las concentraciones en el medio acuífero, debido a la deposición de partículas a través de precipitaciones (Flora, 2015).

1.3.2. Vías de exposición

1.3.2.1 Exposición en población general

Exposición dietética

La principal vía de exposición a los diferentes compuestos de As es a través de la ingestión. En zonas donde los niveles de As en agua subterránea son altos, ya sea por procesos naturales o antropogénicos, la principal vía de exposición al iAs es a través del consumo de agua potable. En ciertas áreas de países como Bangladesh, India, Vietnam, China, Argentina, Chile, México, Australia y EEUU, los niveles de As en agua de consumo se encuentran por encima del valor máximo recomendado por la Organización Mundial de la Salud en el año 2003 (10 µg/L) (Huang et al., 2015; Shaji et al., 2021; World Health Organization, 2017). No obstante, una reciente revisión ha puesto de manifiesto que áreas concretas de hasta 107 países en el mundo pueden estar afectadas por la contaminación de As en agua subterránea, sobre todo en zonas cercanas a cuencas sedimentarias (Shaji et al., 2021). Sin embargo, en muchas otras zonas, los niveles de As en agua potable son muy bajos. Por ejemplo, en España el valor medio cuantificado de As en agua de consumo es de alrededor 1 µg/L, siendo menos de un 1% las determinaciones en agua de consumo >10µg/L (Ministerio de Sanidad Servicios Sociales e Igualdad, 2019a). No obstante, se han identificado algunas zonas en Castilla-León, Galicia, Baleares y Canarias con niveles mayores a los permitidos (Ministerio de Sanidad Servicios Sociales e Igualdad, 2019b).

En regiones donde los niveles de As en el agua potable son bajos, la principal vía de exposición al iAs es a través del consumo de ciertos alimentos, sobre todo cereales, y más específicamente, arroz. El cultivo de arroz se realiza principalmente bajo condiciones anaeróbicas (suelos inundados), donde predominan las formas más móviles de As que se generan bajo ambientes reductores (As^{III}). Este factor parece que está implicado en la bioacumulación de As del suelo en los brotes y el grano, en mayor medida (hasta diez veces más) que otros cereales que crecen en condiciones aeróbicas (Chen et al., 2017; Williams et al., 2007; Xu et al., 2008). Además de la técnica de cultivo, los niveles de As presentes en el arroz muestran variaciones según el tipo de arroz. Parece que las concentraciones de iAs son significativamente más altas en el grano de arroz integral que en los tipos vaporizado y pulido (arroz blanco) (Signes-Pastor et al., 2016; Upadhyay et al., 2019). Aunque el iAs es la especie predominante en el arroz, también se encuentran compuestos metilados, como DMA y MMA, aunque este último en concentraciones mucho

menores (Meharg y Zhao, 2012; Signes-Pastor et al., 2016; U.S Food and Drug Administration, 2016).

Recientemente, la EFSA (2021) ha evaluado la exposición dietética a iAs en la población europea, situándose de media entre 0,03 y 0,15 µg/kg de peso/día en adultos, y entre 0,12 y 0,61 µg/kg de peso/día en niños menores de 36 meses, siendo este último el grupo de población que presentó una exposición más alta. El arroz se sitúa como uno de los mayores contribuidores a la exposición dietética al iAs en ciertos países europeos, como Reino Unido, Suecia y España, debido a sus patrones dietéticos (Food and Agriculture Organization of the United Nations, 2017). En el año 2015 la Comisión Europea reguló el contenido máximo de iAs en el arroz y los productos basados en este alimento, entre 0,20 y 0,30 mg/kg según el tipo de arroz, limitando aún más el contenido en el arroz destinado a la producción de alimentos para lactantes y niños de corta edad (máximo 0,10 mg/kg) (European Commission, 2015b).

Aunque el arroz y otros cereales son los mayores contribuidores a la ingesta de iAs en población general, el consumo de otros productos también contribuye a la exposición dietética total, aunque en proporción sensiblemente menor. Entre estos productos se encuentran los vegetales, las frutas o los productos lácteos (Agència Catalana de Seguretat Alimentaria, 2020; European Food Safety Authority, 2014; European Food Safety Authority et al., 2021).

Finalmente, es importante señalar la contribución del consumo de pescado y marisco en la exposición a As total, sobre todo en áreas donde al consumo de este alimento es frecuente (Agence nationale de sécurité sanitaire de l'alimentation de l'environnement et du travail, 2011; Agència Catalana de Seguretat Alimentaria, 2020). Sin embargo, la principal especie de As presente en este tipo de alimento es la arsenobetaína (AB), considerada como no tóxica para el ser humano (European Food Safety Authority, 2009). Otros compuestos complejos de As presentes en el pescado y marisco son los arsenoazúcares, principalmente en algas marinas, y los arsenolípidos, asociados con el pescado azul (Taylor et al., 2017). Los niveles de iAs en este tipo de productos son generalmente muy bajos, excepto en moluscos y algas (Cheyns et al., 2017; Ferrante et al., 2019). Recientemente, la EFSA está en proceso de evaluar los riesgos de la exposición a especies de As metilado y especies orgánicas de As en los alimentos, en particular la arsenobetaína, los arsenolípidos y los arsenoazúcares (EFSA, 2021).

Exposición inhalada y dérmica

La exposición de As a través de la vía inhalatoria en población general constituye un porcentaje muy bajo respecto al total de la exposición. En el año 2018, menos del 1% de las estaciones de medida de calidad del aire en países europeos comunicaron valores de As por encima del límite establecido por la UE (6ng/m^3) (European Commission, 2004), y todas ellas se encontraban

situadas en áreas industriales suburbanas y de fondo urbano (European Environment Agency, 2020). La población fumadora, sin embargo, sí que parece estar más expuesta a través de esta vía, ya que el As, especialmente los compuestos inorgánicos, son un componente del tabaco (Agency for Toxic Substances and Disease Registry, 2007; Campbell et al., 2014).

La exposición por vía dérmica es rara y ha sido poco estudiada. En estudios experimentales se ha observado que la absorción por esta vía parece ser muy baja (Agency for Toxic Substances and Disease Registry, 2007).

Exposición prenatal

El As es capaz de atravesar la barrera placentaria, y ser transferido de la madre al feto durante la etapa gestacional (Hall et al., 2007; Punshon et al., 2015). Diversos estudios han analizado la presencia de As total, así como de formas inorgánicas y metiladas, en placenta y sangre de cordón umbilical (Freire et al., 2019; Hall et al., 2007; Punshon et al., 2016). Además, se ha observado una correlación alta entre las concentraciones de As medidas en sangre materna y en cordón umbilical (Röllin et al., 2017), lo que indica una eficiente transferencia de este metal entre la madre y el feto.

1.3.2.2 Exposición ocupacional

Exposición inhalada y dérmica

La principal vía de exposición en el ámbito ocupacional es a través de la inhalación de partículas que contienen As, generalmente en combinación con otros elementos. Esta exposición ocupacional puede ser importante en ciertas industrias, como la fundición no ferrosa, y actividades industriales, como la conversación de madera, la desecación de algodón o la fabricación de vidrio (Agency for Toxic Substances and Disease Registry, 2007; European Chemical Agency, 2017; Fowler et al., 2015). Además, los trabajadores que llevan a cabo otras actividades, como aquellos involucrados en funciones de fundición y minería, o en la industria electrónica (esta última por el uso de arseniuro de galio) también pueden estar expuestos a niveles mayores de As (Agency for Toxic Substances and Disease Registry, 2007; Fowler et al., 2015).

Como se ha comentado, la vía dérmica es muy poco frecuente. En el entorno ocupacional únicamente se ha descrito irritación tras el contacto dérmico con polvo que contenía As (European Chemical Agency, 2017).

1.3.3. Toxicocinética del arsénico

Una vez ingerido, el iAs procedente del agua y los alimentos es absorbido, principalmente a través del tracto gastrointestinal, y es transportado a los órganos a través de la sangre, observándose una mayor concentración de este compuesto en el hígado (ver **Figura 4**) (Agency for Toxic Substances and Disease Registry, 2016). Es en este órgano donde se produce la mayor parte de la biotransformación del iAs. El mecanismo metabólico y de biotransformación del iAs aún no está completamente entendido. En la actualidad, se han descrito varias secuencias metabólicas (Cullen, 2014), dos de las más importantes son: a) la vía clásica, propuesta por Challenger (1945) basada en la reducción enzimática del arsénico y metilación oxidativa; y b) la más reciente, basada en la formación no enzimática de complejos arsénico-glutatión (As-GSH) (Hayakawa et al., 2005) (Ver **Figura 5**).

En ambas propuestas, el iAs ingerido es metilado, de MMA a DMA. La metilación de los compuestos está catalizada a través de la enzima arsenito metiltransferasa (AS3MT), que utiliza como principal cosustrato (donador de grupo metilo) la S-adenosil metionina (SAM). La biosíntesis de SAM proviene del metabolismo de 1 carbono (OCM, por sus siglas en inglés), el cual depende del folato y otros micronutrientes, como las vitaminas B_6 y B_{12}, la betaína y la colina (Abuawad et al., 2021).

La propuesta de Challenger postula que únicamente las formas trivalentes pueden ser metiladas, por lo que es necesario un proceso previo de reducción de las formas pentavalentes. Se ha propuesto que el glutatión (GSH) es el principal agente reductor de las formas pentavalentes (Vahter, 2002). En el esquema de Hayakawa se sugiere que GSH es un requisito para la metilación del iAs a través de ASM3T, y los complejos arsénicos tri-glutation (ATG) y monometilarsénico diglutatión (MADG) son los substratos necesarios para la enzima ASM3T. Esta propuesta plantea que las especies trivalentes persisten durante la metilación, y que tras este proceso se produce la oxidación de las especies trivalentes a las pentavalentes.

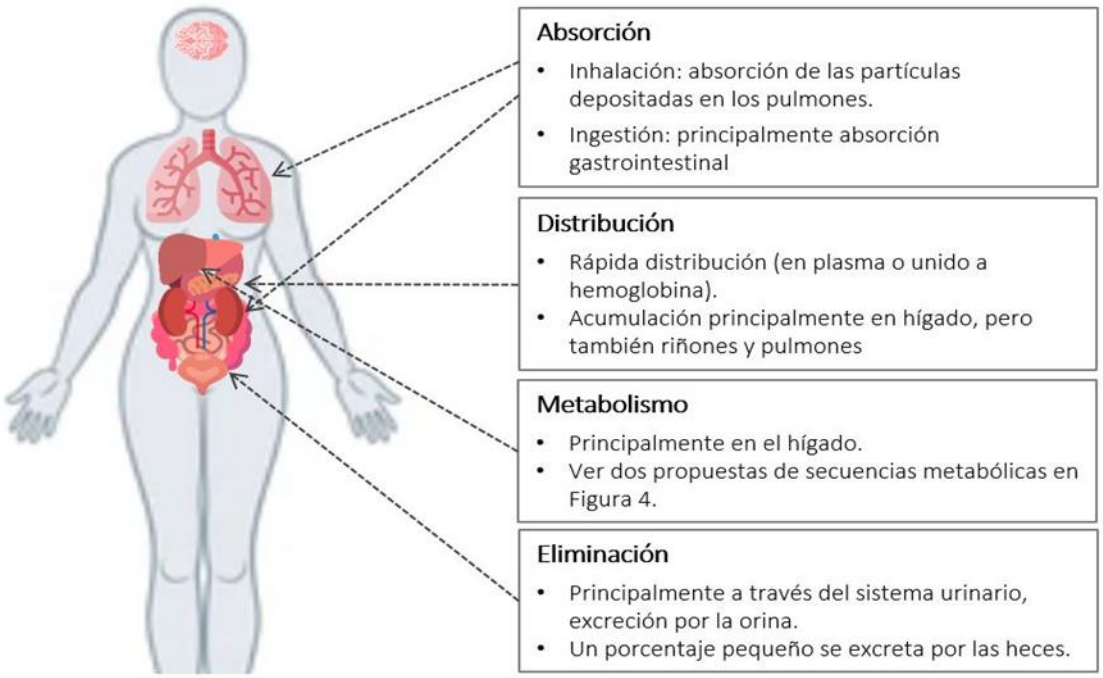


Figura 4 Toxicocinética del arsénico.

Nota: Elaboración propia. Imágenes: shutterstock y Flaticon.

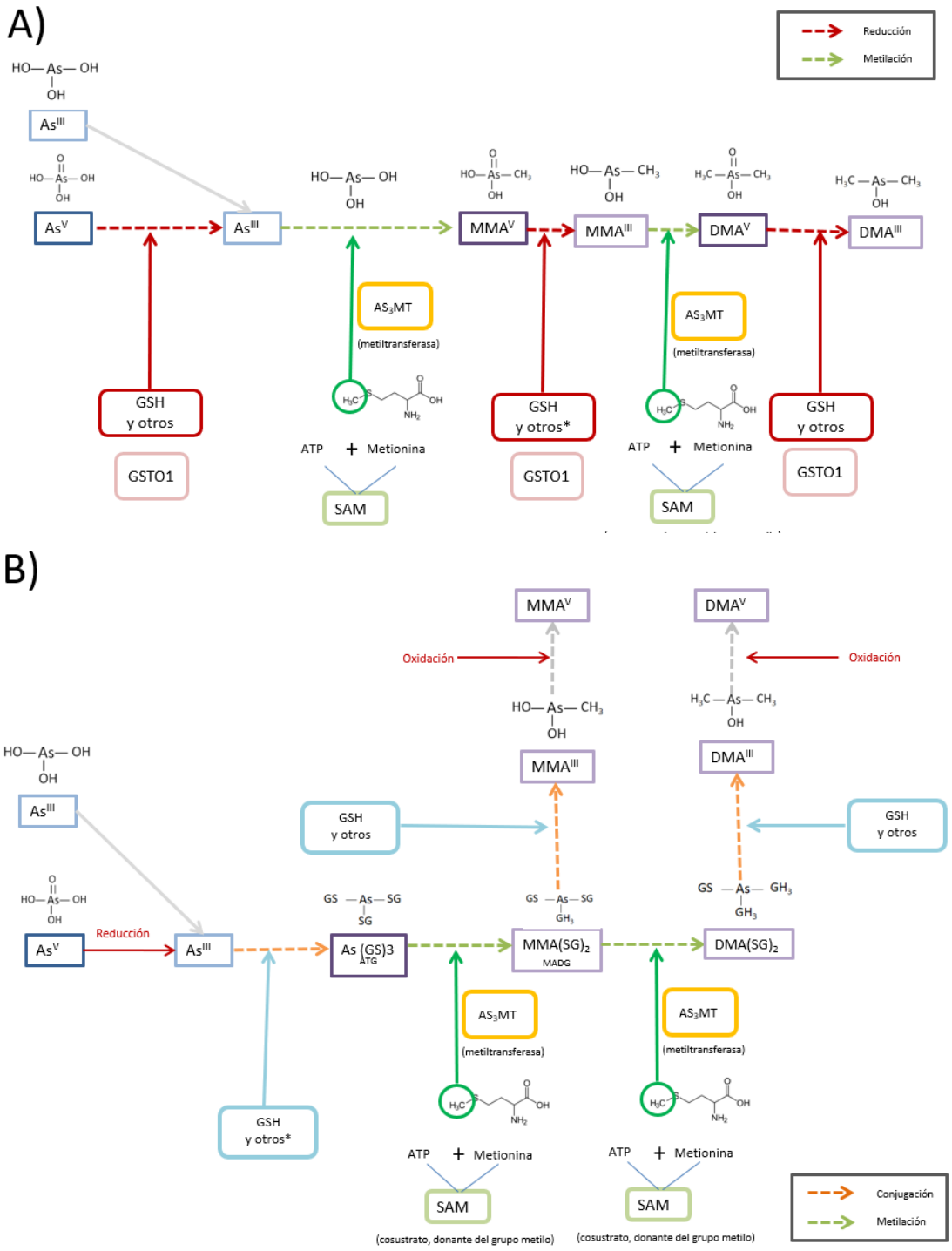


Figura 5. Metabolismo del arsénico inorgánico. A) Reducción y metilación oxidativa (Vía Clásica. Challenger, 1945). B) Formación no enzimática de complejos de arsénico-glutatión (Hayakawa, 2005).

Nota: AS3MT: arsenito metiltransferasa (enzima); ATG: arsénico tri-glutation; GSH: glutatión; GSTO1: Glutatión S-transferasa omega-1 (enzima); MADG: monometilarsénico di-glutatión; SAM= S-adenosil-metionina

Tras este proceso de biotransformación, el iAs y los diferentes metabolitos son excretados a través del sistema urinario. Las proporciones de metabolitos observadas en orina son 60-80% para DMA, 10-20% para MMA y 10-30% para iAs sin metilar (Vahter, 1999). Las concentraciones relativas de los metabolitos en la orina se emplean, actualmente, como un reflejo de la eficiencia en la metilación del iAs, especialmente en poblaciones expuestas a altos niveles de iAs a través del agua potable (Vahter, 1999).

La biotransformación del iAs está considerada un mecanismo de detoxificación. No obstante, se ha demostrado una alta reactividad y toxicidad de compuestos intermedios, como el MMA (Vahter, 2002). Diferentes factores parecen influenciar en la eficiencia en la metilación del iAs, como la edad, el sexo o el consumo de alcohol y tabaco (Shen et al., 2016; Tseng, 2008) o la co-exposición de otros elementos como el selenio (Se), el Mn , el zinc (Zn) o el cadmio (Cd) (G. F. Nordberg et al., 2005; Rahman et al., 2019; H. Sun et al., 2014; Valeri et al., 2017). Ciertos factores nutricionales parecen relacionarse con un metabolismo más eficiente, especialmente aquellos micronutrientes involucrados en el OCM, como las vitaminas B_6 y B_{12}, la betaína, la colina y el ácido fólico (Abuawad et al., 2021; Bozack et al., 2019; Gamble et al., 2006; Heck et al., 2007; Howe et al., 2017; Kurzius-Spencer et al., 2017; Laine et al., 2018; Spratlen et al., 2017). Por último, la gestación parece ser un periodo durante el cual aumenta la eficiencia del metabolismo del iAs, observando un aumento en la excreción de DMA al final del periodo gestacional (Gao et al., 2019; Hopenhayn et al., 2003).

Respecto a las formas más complejas de As, la AB parece excretarse sin cambios a través de la orina. Sin embargo otros compuestos, como los arsenoazúcares y los arsenolípidos parece que son metabolizados, produciendo DMA (European Food Safety Authority, 2009; Molin et al., 2012; Taylor et al., 2017).

1.3.4. Efectos en salud por exposición a arsénico en población general

El arsénico es un tóxico sistémico capaz de causar efectos adversos en múltiples órganos (Agency for Toxic Substances and Disease Registry, 2007). Numerosos estudios epidemiológicos han evidenciado diferentes efectos sobre la salud por exposiciones tanto agudas como crónicas. Aunque la mayoría de los estudios se han realizado en poblaciones expuestas a altos niveles a iAs, sobre todo a través del agua potable, en los últimos años está aumentando la evidencia de efectos relacionados con exposiciones a niveles más bajos. A continuación, se describen los efectos que se han relacionado con la exposición a este tóxico.

Cáncer: en el año 2009, la International Agency for Research on Cancer (IARC) clasificó el As y los compuestos de iAs como carcinógenos para el ser humano (International Agency for Research on

Cancer, 2012). La exposición a este compuesto se ha relacionado con un incremento del riesgo de cáncer de piel, pulmón, vejiga, riñón próstata e hígado (Boffetta y Borron, 2019; Saint-Jacques et al., 2014). Además, varios metaanálisis de estudios epidemiológicos obtuvieron como resultado una relación directa entre el %MMA en orina y el riesgo de padecer cáncer de vejiga, urotelial y de pulmón. De la misma manera, esta relación fue inversa entre el %DMA y el riesgo de desarrollar cáncer de pulmón (Di Giovanni et al., 2020; Gamboa-Loira et al., 2017).

Enfermedades cardiovasculares: se ha observado un aumento del riesgo de desarrollar enfermedades cardiovasculares, coronarias, ictus y enfermedad arterial periférica, así como un aumento en la mortalidad por patología cardiovascular en poblaciones con una alta exposición a As a través del agua potable (>50-150 µg/L) (Moon et al., 2012; Tsuji et al., 2014). A niveles más bajos, se ha observado una relación con la incidencia de patologías cardiovasculares y coronarias (Moon et al., 2017). Un reciente metaanálisis ha evidenciado un aumento del riesgo de enfermedades cardiovasculares en exposiciones por debajo del límite establecido por la OMS (10 µg/L) (Xu et al., 2020). Por su parte, en un estudio longitudinal se asoció una baja eficiencia en la metilación del As (indicada por mayores %MMA y menores %DMA) con un aumento del riesgo de padecer enfermedad coronaria (Chen et al., 2013). Finalmente, se ha evidenciado un aumento de la presión arterial sistólica y de las concentraciones de lipoproteína de baja densidad (LDL) relacionada con mayores concentraciones de As (Zhao et al., 2021; Zhao et al., 2021).

Enfermedades y síntomas respiratorios: se ha observado una relación dosis-respuesta entre la exposición a As (medido en agua potable y en orina) y un déficit en la función pulmonar. Así mismo se ha observado que la exposición a As aumenta la probabilidad de padecer síntomas respiratorios, como tos, disnea, asma y sibilancias (Sanchez et al., 2016).

Diabetes: en este caso, los resultados de una revisión sistemática muestran una asociación entre una mayor eficiencia en la metilación del As (menor %MMA y mayor %DMA) y un aumento del riesgo de desarrollar diabetes o síndrome metabólico (Kuo et al., 2017)

Lesiones en la piel: se ha descrito una relación directa entre la exposición a As y la aparición de lesiones en la piel. Así mismo, una peor eficiencia en la metilación del iAs (mayor %MMA y/o menor %DMA) se ha asociado con el desarrollo de este tipo de lesiones (Karagas et al., 2015).

Efectos neurológicos: estudios transversales han observado efectos adversos en la función sensorial, así como un mayor riesgo de padecer neuropatía periférica (Agency for Toxic Substances and Disease Registry, 2016). Además, un estudio realizado en EEUU relacionó la exposición a As con peores puntuaciones en el lenguaje, función ejecutiva, cognición global y velocidad de procesamiento entre otros. No obstante, la evidencia sobre de la exposición de As y efectos neurológicos en adultos es todavía limitada (O'Bryant et al., 2011).

1.3.5. Efectos en salud por exposición a arsénico durante etapas vulnerables

1.3.5.1 Efectos en el desarrollo neuropsicológico por exposición temprana a As

Solo unos pocos estudios han evaluado con un diseño longitudinal los efectos de la exposición a As *in utero* y el desarrollo neuropsicológico infantil, obteniendo resultados heterogéneos. En un estudio llevado a cabo en Bangladesh se encontró una relación negativa entre el As total urinario materno a las 8 y 30 semanas de gestación (en este estudio, el As total es la suma de iAs, MMA y DMA, excluyendo otras formas complejas de As) y las escalas verbal y total de la Escala de Inteligencia Preescolar y Primaria de Wechsler a los 5 años. Sin embargo, esta relación únicamente fue significativa en niñas (Hamadani et al., 2011). En la misma cohorte, el As prenatal se asoció de manera negativa con las escalas de comprensión verbal, velocidad de procesamiento, razonamiento de procesamiento y la puntuación total de desarrollo, evaluado mediante la Escala de Inteligencia de Wechsler para niños y niñas a los 10 años de edad. No obstante, el efecto en estas dos últimas escalas únicamente fue significativo en niñas (Vahter et al., 2020).

Un estudio de cohortes realizado en Nepal no encontró evidencia de asociación entre el As total de la sangre del cordón umbilical y el neurodesarrollo infantil al nacimiento, 6, 24 y 36 meses de edad (Parajuli et al., 2013, 2014; Parajuli et al., 2015a; Parajuli et al., 2015b). Por otro lado, en un estudio realizado en población española observó una asociación negativa entre los niveles de As total placentario y las puntuaciones en las escalas ejecutiva y verbal de las Escalas McCarthy de Habilidades Infantiles (MSCA) (Freire et al., 2018). En todos los estudios el biomarcador utilizado para evaluar la exposición fue el As total (suma de formas orgánicas e inorgánicas) o la suma de las concentraciones de iAs y las formas metiladas (DMA y MMA).

Respecto a la exposición postnatal a As, los posibles efectos en el neurodesarrollo infantil han sido igualmente evaluados durante las últimas dos décadas, especialmente en áreas con una alta exposición a As a través del agua potable. Los resultados de estos estudios, la mayoría de diseño transversal, son también poco consistentes. En estudios realizados en Bangladesh (Parvez et al., 2011; Wasserman et al., 2011, 2016, 2018), India (Von Ehrenstein et al., 2007) y México (Calderón et al., 2001; Rocha-Amador et al., 2007; Rosado et al., 2007) se ha observado una relación inversa entre los niveles de As medidos en sangre y diferentes funciones motoras y cognitivas durante la etapa escolar y la adolescencia. Otros estudios no encontraron ninguna relación entre la exposición postnatal a As y el neurodesarrollo infantil. Por ejemplo, en el estudio de Wasserman et. al (2014), realizado en Maine (EEUU), no se observó ninguna relación entre las

concentraciones de As total medido en uñas y el desarrollo cognitivo (inteligencia). Finalmente, un estudio realizado en Uruguay, no encontró ninguna asociación significativa entre el As total medido en orina y ninguna de las escalas de evaluación cognitiva en niños y niñas entre 5 y 8 años (Desai et al., 2018).

De la misma manera que en la evaluación de la exposición prenatal a As, el biomarcador utilizado en todos los estudios presentados es As total medido generalmente en orina. Sin embargo, en un estudio realizado en España con una submuestra de niños y niñas de la cohorte INMA se analizó la suma de DMA, MMA e iAs (∑As) en muestras de orina recogidas a los 4 años. Aquí se observó una asociación negativa con las escalas motora global, motora fina y de memoria de trabajo (en niños) y motora gruesa (en niñas) (Signes-Pastor et al., 2019).

1.3.5.2 Otros efectos en salud por exposición temprana a As

Además de efectos en el desarrollo neuropsicológico, se ha observado que la exposición prenatal y postnatal a As puede tener un impacto en otros resultados en salud durante la infancia.

Enfermedades y síntomas respiratorios: la exposición a As durante el embarazo se ha asociado con un aumento en la presencia de síntomas respiratorios (sibilancias, asma, tos y disnea) durante la infancia y la edad adulta. Así mismo, también se ha relacionado con un aumento de las infecciones respiratorias en los primeros años de vida (Sanchez et al., 2016; Signes-Pastor, Martinez-Camblor, et al., 2021). Otros efectos negativos sobre la función respiratoria también se ha relacionado con la exposición a As durante la infancia, como la aparición de síntomas respiratorios (por ejemplo, tos), así como un mayor riesgo de padecer rinitis alérgica; sin embargo la evidencia es menos concluyente (Sanchez et al., 2016).

Resultados adversos gestacionales: un metaanálisis de estudios epidemiológicos ha observado un incremento del riesgo de aborto espontáneo y muerte fetal, especialmente relacionado con exposiciones altas a As a través del agua potable (>50µg/L) (Quansah et al., 2015).

Antropometría al nacimiento y durante la infancia: la exposición materna a As durante el embarazo, sobre todo a niveles altos, parece relacionarse con una disminución del peso y la talla al nacimiento. También se ha observado una disminución del perímetro cefálico (Quansah et al., 2015; Zhong et al., 2019). Respecto a la exposición postnatal, en dos estudios realizados en Bangladesh se observó una relación inversa entre la exposición postnatal a As y el peso y la talla, especialmente en niñas, así como un mayor riesgo de bajo peso (Alao et al., 2021; Gardner et al., 2013).

1.3.6. Niveles de referencia y valores límite de As

Actualmente no existe una ingesta tolerable de As y/o iAs establecida. En el 2009, la EFSA hizo una revisión sobre la exposición a As en Europa y publicó un informe concluyendo que la ingesta semanal tolerable provisional establecida en 1988 por el Comité Mixto FAO / OMS de Expertos en Aditivos Alimentarios (JECFA) de 15 µg/kg/peso corporal no era apropiada. En el mismo informe, el Panel de la EFSA sobre contaminantes en la cadena alimentaria (Panel CONTAM) estableció una dosis de referencia de exposición (benchmark dose lower level [BMDL01], para un aumento del 1% en el riesgo de cáncer de pulmón, piel y vejiga, así como de lesiones cutáneas) de entre 0,3 y 8 µg/kg de peso corporal/día (European Food Safety Authority, 2009). Posteriormente, JECFA estableció un BMDL05 de 3,0 µg/kg de peso corporal/día (rango 2-7) para un incremento del cáncer de pulmón (Joint FAO/WHO Expert Committee on Food Additives, 2011) (Tabla 2) .

Por su parte, con el objetivo de reducir la exposición a este tóxico, la Comisión Europea ha establecido unos límites máximos de As en arroz y productos derivados de este alimento (Tabla 2). Así mismo, el Consejo de la Unión Europea estableció en 1998, y consolidó en 2015, un máximo de 10 µg/L de As en agua de consumo humano (Council of the European Union, 2015), siendo el mismo valor máximo recomendado por la Organización Mundial de la Salud en el año 2003 (World Health Organization, 2017).

Finalmente, la Agencia Europea de Sustancias Químicas estableció un valor de referencia biológica (BGV) para ámbitos ocupacionales de 10 µg/L en orina basado en el percentil 95 de los datos de la población general establecidos para la suma de iAs, DMA y MMA (European Chemical Agency, 2017). Este mismo valor ha sido establecido por el Instituto Nacional de Seguridad Salud y Bienestar en el Trabajo en España (Instituto Nacional de Seguridad Salud y Bienestar en el Trabajo, 2018)

Valores Guía máximo de As en agua de consumo humano[1]	
10 µg/L	
Niveles máximos de As inorgánico en productos alimenticios[2]	
Arroz elaborado (arroz pulido o blanco), no sancochado	0,20 mg/kg
Arroz sancochado y arroz descascarado	0,25 mg/kg
Tortitas, obleas, galletitas y pasteles de arroz	0,30 mg/kg
Arroz destinado a la producción de alimentos para lactantes y niños de corta edad	0,10 mg/kg
Referencia de ingesta diaria de As inorgánico ($BMDL_{0.5}$)[3]	
3 µg/kg peso corporal/ día (rango 2,7-7,0)	
Valor límite ambiental de exposición laboral de arsénico* inhalable[4]	
0,01 mg/m3	
Valor límite biológico de As elemental y compuestos inorgánicos solubles [4]	
35 µg As/ l (iAs + DMA+MMA en orina)	

Tabla 2. Niveles de referencia y valores límites de As.

Nota: [1] Council Directive 98/83/EC, 2015; OMS, 2017; [2] Comisión Europea, 2015; [3] JEFSA, 2011; [4]Instituto Nacional de Seguridad Salud y Bienestar en el Trabajo, 2018.
* Ácido arsénico y sus sales, As elemental, compuestos inorgánicos solubles de As.

1.4. EL PROYECTO INFANCIA Y MEDIO AMBIENTE (INMA)

La presente tesis doctoral se enmarca en el Proyecto INMA (http://www.proyectoinma.org/). Esta red de investigación cooperativa se constituyó en el año 2003. Los principales objetivos del proyecto son:

- Describir el nivel de exposiciones prenatales individuales a contaminantes ambientales y las dosis internas de estos contaminantes durante la gestación, el nacimiento y durante la infancia hasta la adolescencia en España.
- Evaluar el impacto de la exposición pre y postnatal a diferentes contaminantes ambientales en el crecimiento, la salud y el desarrollo de los niños y las niñas, desde las etapas tempranas fetales y a lo largo de su vida.
- Evaluar cómo los factores genéticos y nutricionales pueden modificar los efectos de los contaminantes ambientales en la salud y el crecimiento infantil.

Esta red está formada por siete cohortes (Ribera d´Ebre, Menorca, Granada, Sabadell, Asturias, Gipuzkoa y Valencia), en las cuales participan más de 3900 mujeres embarazadas y sus recién nacidos (Guxens et al., 2012) **(Figura 6**). Los criterios de inclusión fueron: pertenecer al área de estudio de cada cohorte, tener un mínimo de 16 años, tener un embarazo único, no haber seguido ningún programa de reproducción asistida, intención de continuar el seguimiento y dar a luz en el hospital de referencia y no tener problemas de comunicación.

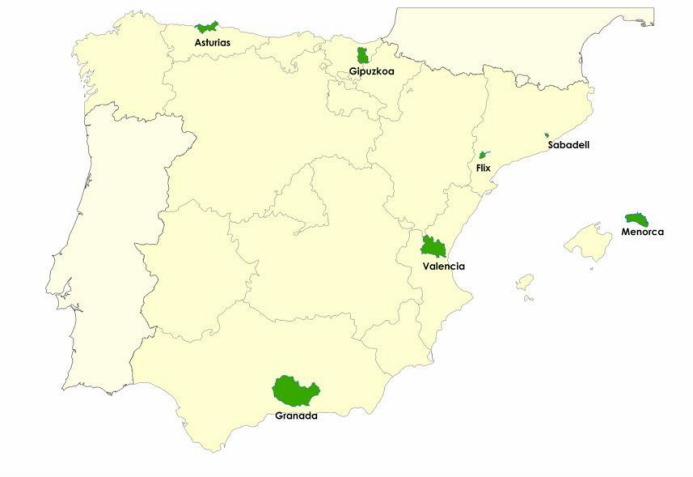


Figura 6. Localización de las áreas de estudio de las cohortes del Proyecto INMA.
Nota: elaboración propia. Fuente: adaptado de www.proyectoinma.org

En todas las cohortes participantes se realizó un seguimiento prospectivo de las mujeres embarazadas hasta el momento del parto. Con el nacimiento, sus hijos e hijas comenzaron a formar parte de la cohorte de participantes. Durante las visitas de evaluación realizadas a lo largo de la infancia (nacimiento, 6 y 14 meses y 4-5, 7-8, 9-10, 11-12 y 14-16 años) se han evaluado diferentes aspectos del desarrollo y salud infantil. Durante estas visitas se han recogido muestras biológicas y ambientales, además de información sociodemográfica, clínica, ambiental, dietética y otros hábitos de vida, a través de cuestionarios y exploraciones físicas (ver **Figuras 7 y 8**). Gracias a esta información ha sido posible plantear, entre otras hipótesis, la influencia de la exposición a metales en el desarrollo fetal y la salud infantil (Guxens et al., 2012).

Para la realización de la presente tesis, los participantes incluidos proceden de las cohortes de Gipuzkoa y Valencia.

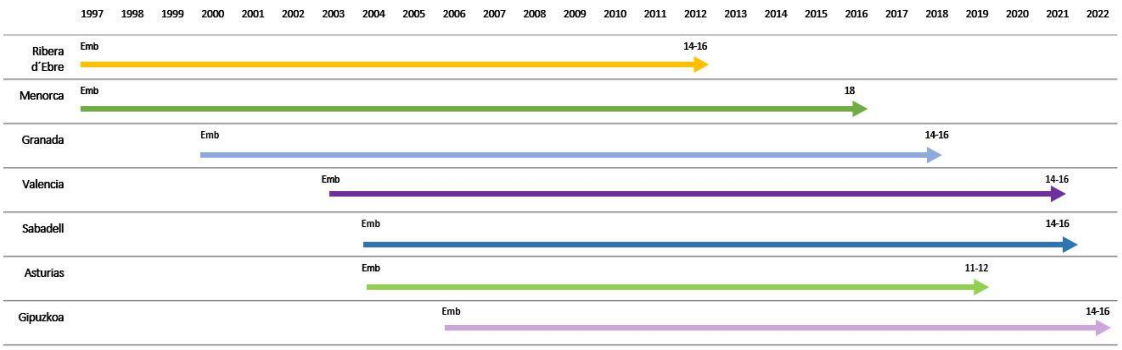


Figura 7. Períodos de seguimiento en las cohortes del Proyecto INMA.
Nota: Elaboración propia. Fuente: www.proyectoinma.org

	Embarazo		Nacim.	1-2 a	4-5 a	7-8 a	9-10 a	11-12 a	14-16 a
	1T	3T							
Cuestionarios									
Muestras biológicas									
Evaluación del neurodesarrollo									
Evaluación respiratoria									
Medidas antropométricas									
Evaluación presión arterial									

Figura 8. Información recogida en cada visita de seguimiento del proyecto INMA.
Nota: Elaboración propia. Fuente: www.proyectoinma.org

CAPÍTULO II. JUSTIFICACIÓN

2. JUSTIFICACIÓN

Tanto el periodo prenatal como los primeros años de vida constituyen una etapa especialmente vulnerable a la exposición a neurotóxicos, incluso a niveles inocuos para los adultos. Los efectos que se producen durante este periodo condicionan el desarrollo y la calidad de vida a lo largo de toda la vida. Además, dicha exposición, podría provocar cambios permanentes que pueden dar lugar a la aparición de efectos adversos durante la vida adulta. Sin embargo, esta etapa vulnerable también es una ventana de oportunidad para poder poner en marcha estrategias de protección, prevención y promoción de la salud que permitan minimizar los posibles efectos sobre el desarrollo neuropsicológico infantil.

Como se ha comentado en la introducción, existe evidencia de que la exposición a As y niveles altos de Mn pueden causar efectos adversos sobre la salud. Diversos estudios epidemiológicos han observado que esta exposición a As durante la etapa prenatal, o a niveles inadecuados de Mn podrían tener un impacto sobre el desarrollo neuropsicológico durante la infancia. Sin embargo, la mayoría de estos estudios se han realizado en áreas donde la exposición ambiental a estos compuestos es alta. Actualmente, se sabe muy poco sobre el efecto que puede producir la exposición prenatal a estos elementos en zonas donde el nivel de exposición ambiental es bajo, como en España.

A la falta de evidencia en áreas de baja exposición se unen problemas o deficiencias metodológicas. Este es el caso de la evaluación de la exposición a As, donde la mayoría de los estudios han evaluado como biomarcador de exposición el As total o la suma de DMA, MMA e iAs, lo que puede llevar a cierta imprecisión en la evaluación, especialmente cuando la mayoría de la exposición es a formas de As consideradas como no tóxicas procedentes del pescado. Además, existen muy pocos estudios que evalúen la eficiencia en la metilación del As y su relación con el desarrollo neuropsicológico infantil. Por su parte, aunque el Mn es un elemento esencial necesario para el correcto funcionamiento y desarrollo fisiológico, la evidencia sobre cuáles son los niveles adecuados a este compuesto es todavía insuficiente. Aumentar el conocimiento en este sentido podría, igualmente, proporcionar información valiosa para detectar grupos de población vulnerables e implementar estrategias de salud pública.

El Proyecto INMA ofrece la oportunidad de ayudar a responder las cuestiones planteadas. Su carácter multicéntrico y longitudinal, así como la exhaustiva recogida de gran variedad de

información durante los diferentes seguimientos, permite estudiar la relación entre la exposición prenatal a estos compuestos y el impacto en el desarrollo neuropsicológico infantil, teniendo en cuenta los diversos factores que puedan estar implicados en dicha relación. Así mismo, este estudio de cohortes nos ofrece la oportunidad de conocer los factores relacionados con la exposición a ambos compuestos durante el periodo prenatal, así como la relación que pueda existir entre ellos.

CAPÍTULO III. HIPÓTESIS Y OBJETIVOS

3.1 HIPÓTESIS

Hipótesis 1: la exposición a Mn durante el embarazo se asocia con un retraso en el desarrollo neuropsicológico infantil.

Hipótesis 2: la exposición a As y a sus diferentes metabolitos durante el embarazo se asocia con un retraso en el desarrollo neuropsicológico infantil.

Hipótesis 3: una peor eficiencia en la metilación del As durante el embarazo se asocia con un retraso en el desarrollo neuropsicológico infantil.

Hipótesis 4: los niveles de ciertos nutrientes y elementos, como el selenio y el hierro, modifican la asociación de la exposición prenatal a Mn sobre el desarrollo neuropsicológico en la infancia.

Hipótesis 5: los niveles de ciertos nutrientes y elementos, como el selenio, zinc, y vitaminas del grupo B, modifican el efecto de la eficiencia en la metilación del arsénico, sobre el desarrollo neuropsicológico en la infancia.

Hipótesis 6: los niveles de Mn durante el embarazo modifican la asociación de la exposición prenatal a As sobre el desarrollo neuropsicológico en la infancia.

3.2 OBJETIVOS

3.2.1 Objetivo general

Estudiar la relación entre la exposición prenatal a Mn y a As y la aparición de efectos adversos en el desarrollo neuropsicológico infantil en los participantes de las cohortes INMA de Valencia y Gipuzkoa.

3.2.2 Objetivos específicos

Objetivo específico 1: describir las concentraciones de manganeso en muestras de suero materno recogidas durante el primer trimestre del embarazo.

Objetivo específico 2: estudiar los factores sociodemográficos, ambientales y dietéticos que se relacionan con las concentraciones de manganeso durante el embarazo.

Objetivo específico 3: evaluar la relación entre las concentraciones prenatales de manganeso y el desarrollo neuropsicológico de los niños y niñas al año de edad.

Objetivo específico 4: estudiar la modificación de efecto producida por el sexo, los niveles maternos de ciertos elementos, así como la ingesta de vitaminas durante el embarazo en la relación entre la exposición prenatal a manganeso y el desarrollo neuropsicológico de los niños y niñas al año de edad.

Objetivo específico 5: describir las concentraciones de arsénico total y sus diferentes metabolitos (AB, DMA, MMA e iAs) en muestras de orina materna recogida durante el primer trimestre del embarazo.

Objetivo específico 6: analizar y describir la eficiencia en la metilación del arsénico durante el primer trimestre del embarazo.

Objetivo específico 7: estudiar los factores sociodemográficos, ambientales y dietéticos que se relacionan con las concentraciones de arsénico y sus diferentes metabolitos durante el embarazo.

Objetivo específico 8: estudiar los factores sociodemográficos, ambientales y dietéticos que se relacionan con la eficiencia en la metilación del As durante el embarazo.

Objetivo específico 9: evaluar la relación entre las concentraciones prenatales de arsénico y sus metabolitos y el desarrollo neuropsicológico de los niños y niñas a los 4-5 años de edad.

Objetivo específico 10: evaluar la relación entre la eficiencia en la metilación del arsénico en el embarazo y el desarrollo neuropsicológico de los niños y niñas a los 4-5 años de edad.

Objetivo específico 11: estudiar la modificación de efecto producida por el sexo, los niveles maternos de ciertos nutrientes y elementos (selenio, hierro, zinc, y ciertas vitaminas del grupo B), y la exposición a otros metales tóxicos (cadmio y mercurio) en la relación entre la eficiencia en la metilación del arsénico y el desarrollo neuropsicológico de los niños y niñas al 4-5 años de edad

Objetivo específico 12: estudiar la modificación de efecto producida los niveles maternos de manganeso en la relación entre la exposición prenatal a arsénico y sus metabolitos, así como la eficiencia en la metilación del arsénico y el desarrollo neuropsicológico de los niños y niñas al 4-5 años de edad

CAPÍTULO IV. METODOLOGÍA GENERAL

4.1 POBLACIÓN DE ESTUDIO

La población de estudio incluyó las mujeres embarazadas reclutadas en las cohortes de Valencia (años de reclutamiento: 2003-2005) y Gipuzkoa (años de reclutamiento: 2006-2008), y sus hijos e hijas. Para el reclutamiento en ambas cohortes se utilizó un protocolo común. Las mujeres fueron captadas mediante muestreo consecutivo durante la primera visita de control del embarazo en el hospital. Se incluyeron todas aquellas mujeres que aceptaron participar y que cumplían los criterios de inclusión: tener al menos 16 años, embarazo único, sin tratamiento de fertilización para el presente embarazo, no presentar problemas para la comunicación y que tuvieran previsto realizar el seguimiento programado y dar a luz en dicho hospital. Se reclutaron 1493 mujeres que fueron monitorizadas durante el embarazo hasta el momento del parto, a partir de este momento fueron sus hijos e hijas los que se incluyeron en la cohorte de nacimiento (n=1399).

La información sobre el seguimiento y flujo de participantes, pérdidas y tamaño muestral total y por cohorte/área de estudio para cada uno de los artículos que componen la tesis puede consultarse en la Figura 9.

El protocolo de estudio fue aprobado por el Comité de Ética del Hospital Universitario La Fe, el Comité de Ética del Centro Superior de Investigación en Salud Pública de Valencia (Valencia) y el Comité de Ética del Hospital Donostia (Gipuzkoa). El consentimiento informado con respecto al período prenatal fue firmado por la madre y en cada fase del período posnatal se firmó el consentimiento adicional por uno de los padres o un representante legal.

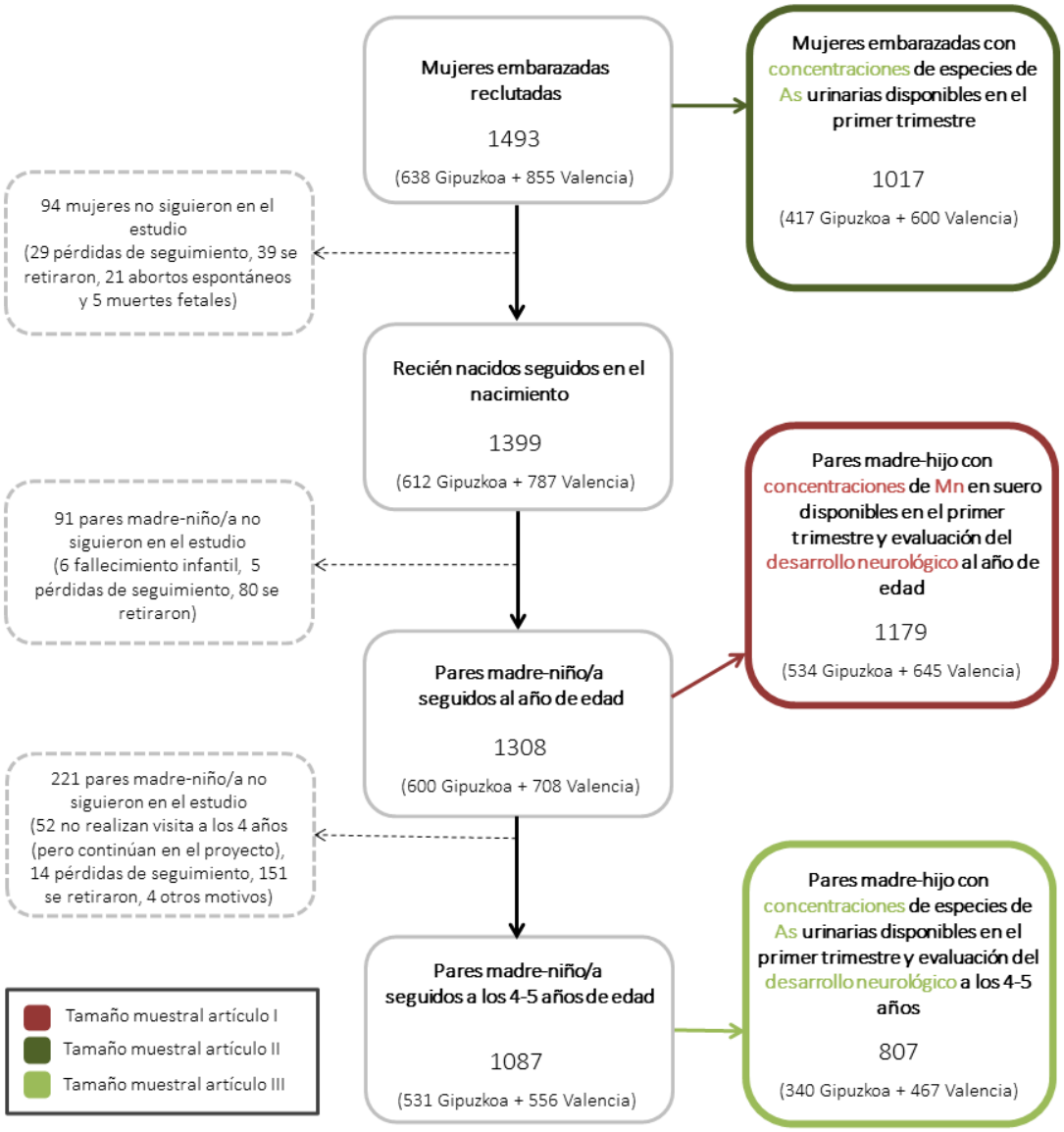


Figura 9. Diagrama de flujo del seguimiento de las personas participantes y tamaño muestral en cada uno de los estudios que forman la presente tesis.

4.2 VARIABLES DE ESTUDIO

4.2.1 Variables de exposición

4.2.1.1 Análisis del manganeso prenatal (Artículo II)

Se midió la concentración de Mn en muestras de suero materno recogidas al final del primer trimestre del embarazo. Tras la extracción de sangre venosa y posterior separación del suero mediante centrifugación, las muestras se almacenaron a -80ºC y se transportaron congeladas al Instituto Karolinska (Suecia) para su análisis. Las muestras de suero se prepararon para su análisis mediante un método de dilución alcalina directa (Levi et al., 2018). Las concentraciones de Mn en suero se determinaron mediante espectrometría de masas de plasma acoplado inductivamente (ICPMS; Agilent 7700x, Agilent Technologies, Tokio, Japón) equipado con un sistema de reacción octopolar con tecnología de células de colisión/reacción. El límite de detección (LOD) para las muestras de Valencia fue de 0,37 µg/L y para las muestras de Gipuzkoa 0,45 µg/L. Ninguna de las muestras analizadas presentaron concentraciones por debajo del LOD. La exactitud y precisión de cada análisis se verificó analizando materiales de referencia disponibles comercialmente. Se observaron variaciones en las concentraciones de Mn relacionadas con el día de análisis de laboratorio, por lo que se estandarizaron los niveles de acuerdo con esta variable.

4.2.1.2 Análisis y especiación del arsénico prenatal (Artículo II y III)

Las concentraciones de As total y sus metabolitos se determinaron en muestras de orina recolectadas en el primer trimestre del embarazo. Tras su recolección, las muestras de orina se mantuvieron congeladas a -80 ° C hasta el momento de ser transportadas al Instituto de Química de la Universidad de Graz (Austria) para su análisis.

Las concentraciones de As total se determinaron con un espectrómetro de masas en tándem de plasma acoplado inductivamente (ICPMS/MS, 8800, Agilent Technologies, Waldbronn, Alemania) con oxígeno como gas de reacción a m/z 75 → 91. La separación cromatográfica de los compuestos de As se realizó de acuerdo con un método previamente validado (Scheer et al., 2012). Para la especiación de As se empleó cromatografía líquida de alta resolución (HPLC, 1200, Agilent Technologies) acoplada a ICPMS/MS (8800, Agilent Technologies).

Los LOD de AB, DMA, MMA e iAs para las muestras de Valencia fueron de 0,02, 0,02, 0,03 y 0,03 µg/L y para las muestras de Gipuzkoa fueron de 0,02, 0,03, 0,03 y 0,02 µg/L. Cuando las

muestras estaban por debajo del LOD, se asignó ½ LOD para el análisis estadístico (2,8% de las muestras para los niveles de MMA y 2,3% de las muestras para los niveles de iAs).

4.2.2 Variables de resultado

4.2.2.1 Evaluación del desarrollo neuropsicológico a los 12-14 meses del nacimiento (Artículo I)

El desarrollo cognitivo y psicomotor se evaluó mediante las Escalas de Desarrollo Infantil de Bayley (BSID) versión I (Bayley, 1977), compuestas por las escalas mental y psicomotora. La escala mental evalúa el desarrollo mental apropiado para la edad en áreas cognitivas como la capacidad de desempeño, la memoria y el primer aprendizaje verbal. La escala psicomotora evalúa el desarrollo psicomotor fino y grueso. Puntuaciones más altas en las escalas indican un mejor desarrollo. Todas las pruebas fueron realizadas por psicólogos capacitados en presencia de su madre. Para limitar la variabilidad interobservador, se aplicó un protocolo estricto, que incluyó sesiones de entrenamiento donde se cuantificaron las diferencias interobservador y el uso de tres conjuntos de controles de calidad (pruebas de confiabilidad interobservador). La confiabilidad entre evaluadores estimada por correlación intraclase fue 0,90 para las puntuaciones de las pruebas mentales y 0,91 para las puntuaciones de las pruebas psicomotoras. Las puntuaciones se estandarizaron por la edad del niño (días) en el momento de administración de la prueba y por psicólogo. A continuación, se tipificaron los residuos estandarizados teniendo una media de 100 puntos (desviación estándar [DE]= 15) para homogeneizar las escalas.

4.2.2.2 Evaluación del desarrollo neuropsicológico a los 4-5 años (Artículo III)

El neurodesarrollo a los 4-5 años de edad se evaluó a través de las Escalas de Habilidades Infantiles McCarthy (MCSA) adaptadas a la población española (McCarthy, 2009), compuestos por una escala global y cinco escalas de subáreas. La escala verbal se refiere a tareas cognitivas relacionadas con el procesamiento de información verbal; la escala de habilidades numéricas; la escala perceptivo-manipulativa se refiere a las tareas cognitivas relacionadas con el procesamiento de la información perceptiva, incluida la ejecución manual; la escala de memoria considera la retención de información a corto plazo (verbal, visual o numérica); y la escala motora se refiere a habilidades finas (por ejemplo, dibujo) y gruesas (por ejemplo, equilibrio o precisión). La suma de las tres primeras escalas proporciona la escala general cognitiva. Además, se utilizaron cuatro subáreas de nueva construcción para evaluar las tareas cognitivas asociadas a la función ejecutiva (motricidad gruesa y fina, función ejecutiva y memoria de trabajo) (Julvez et al., 2011). Puntuaciones más altas en la escala general y las subescalas indican un mejor desarrollo.

Todas las pruebas fueron realizadas por psicólogos capacitados. Para limitar la variabilidad interobservador, se aplicó un protocolo estricto que incluía entrenamientos interobservadores (variabilidad interobservador evaluada por las correlaciones de Pearson fue inferior <5%). La consistencia interna de las subescalas de MSCA se evaluó utilizando el coeficiente alfa de Cronbach obteniendo valores que van desde 0,64 (motor) a 0,90 (índice cognitivo global). Las puntuaciones se estandarizaron por la edad del niño (días) en el momento de administración de la prueba y por psicólogo. A continuación, se tipificaron los residuos estandarizados teniendo una media de 100 puntos (DE = 15) para homogeneizar las escalas.

4.2.3 Covariables

Las covariables utilizadas en cada artículo pueden consultarse en la Tabla 3.

4.2.3.1 Variables sociodemográficas, ambientales y clínicas obtenidas mediante cuestionario

Durante el embarazo, las mujeres completaron dos cuestionarios en el primer y tercer trimestre de gestación. Los cuestionarios fueron administrados por entrevistadores entrenados. Estos cuestionarios se centraron en información sociodemográfica, ambiental y de estilos de vida durante el embarazo.

De la misma manera, se recogió información mediante cuestionarios sobre variables sociodemográfica, ambiental y de estilos de vida al año y los 4-5 años, coincidiendo con las evaluaciones del desarrollo neuropsicológico.

4.2.3.2 Variables dietéticas

La información dietética durante el embarazo se recogió a través de un cuestionario semicuantitativo de frecuencia alimentaria (CFA) administrado en el primer y tercer trimestre. Este CFA ha sido validado con buena reproducibilidad para la ingesta de nutrientes y alimentos (Vioque et al., 2013). Para los estudios realizados en la presente tesis, únicamente se ha utilizado la información derivada del CFA del primer trimestre, debido a la proximidad temporal a la medición de los biomarcadores de interés (Mn y As). Los ítems constan de nueve respuestas posibles, con un rango desde "*nunca o menos de una vez al mes*" hasta "*seis o más por día*". Cada alimento tiene un tamaño de porción específico, lo que sirve para calcular la ingesta diaria promedio en gramos para cada participante (gramos/día). Los grupos de alimentos utilizados han sido: lácteos, huevos, carnes (en total y categorizada en carne roja y blanca), pescados y mariscos (en total y categorizadas en pescado blanco, pescado azul y marisco y moluscos), frutas, verduras,

legumbres, frutos secos, patatas, cereales y pan, y café y otras infusiones. También se obtuvo información sobre el consumo de agua del grifo. Las ingestas ajustadas por energía se calcularon utilizando el método de residuos (Vioque et al., 2013).

La ingesta dietética de ciertos nutrientes (folato, ácido fólico, vitaminas B_{12} y B_6, Fe y Zn) se estimó utilizando tablas de composición de alimentos (Palma et al., 2008; U.S. Department of Agriculture: Agricultural Research Service USDA, 2007). También se recopiló información sobre la ingesta de suplementos (nombre de marca, dosis y composición), que se convirtió en dosis diaria de ingesta de nutrientes y se agregó al cálculo de la ingesta diaria total de nutrientes.

4.2.2.3 Otros biomarcadores

Las concentraciones de Se en suero materno recogido durante el primer trimestre del embarazo se determinaron mediante espectrometría de masas de plasma acoplado inductivamente con el sistema celular de colisión/reacción en modo hidrógeno. La cuantificación de los niveles plasmáticos maternos de ferritina se realizó mediante fluoroinmunoensayo (DELFIA Ferritin kit A069-101) en la cohorte de Gipuzkoa y mediante inmunoturbidimetría en analizadores Beckman Coulter AU en Valencia. Las concentraciones de Cd y Zn se determinaron mediante ICPMS en muestras de orina recogidas en el primer trimestre del embarazo. Las concentraciones de creatinina se midieron en la misma muestra de orina mediante la prueba DRI® Creatinine-Detected® utilizando AV680 de Beckman Coulter.

4.2.2.4 Evaluación de la exposición a contaminación atmosférica

Se evaluaron las exposiciones ambientales a dióxido de nitrógeno (NO_2) y $PM_{2,5}$ durante el embarazo. No obstante, la información utilizada para la presente tesis se refiere a la exposición durante el primer trimestre del embarazo. Las concentraciones de NO_2 se utilizaron para estimar la exposición individual a la contaminación atmosférica relacionada con el tráfico y se midieron mediante muestreadores pasivos (Radiello, Fundazione Salvatore Maugeri, Padua, Italia) distribuidos según criterios geográficos y de densidad de población. Los muestreadores permanecieron expuestos durante cuatro períodos de muestreo de siete días cada uno. Con los resultados de los captadores pasivos y de datos de sistemas de información geográfica (GIS, por sus siglas en inglés, "*Geographical Information Systems*"): altitud, distancia a carreteras, se construyeron modelos de regresión de usos del suelo (LUR, por sus siglas en inglés, "land use regression") utilizando técnicas de geoestadística para obtener las estimaciones espaciales de contaminantes en las áreas de estudio. Para el cálculo de la exposición individual de cada participante durante el embarazo, se obtuvieron las estimaciones espaciales de NO_2 correspondientes a la residencia de cada mujer participante y se ajustaron temporalmente a su periodo de embarazo utilizando los niveles diarios de NO_2 obtenidos de las estaciones de la red

de monitoreo que cubren el área de estudio. Se puede encontrar más información sobre la metodología de evaluación de exposición a contaminación atmosférica en Iñiguez et.al., (2009) y Estarlich et.al., (2011, 2016).

Además, para la cohorte de Gipuzkoa se evaluó la exposición $PM_{2,5}$. Se realizó la medición durante todo el embarazo mediante tres muestreadores de gran volumen Digitel DHA-80. Las descripciones del área de estudio, la metodología de monitoreo de la contaminación del aire y la cuantificación de partículas de la calidad del aire en el área se pueden consultar en Lertxundi et.al., (2015).

		Artículo I			Artículo II			Artículo III		
		E	R	C	E	R	C	E	R	C
Variables principales de exposición y resultado										
As total y metabolitos (orina)	Continua (µg/L)									
Mn (suero)	Continua (µg/L)									
BSID	Continua									
MSCA	Continua									
Covariables										
Variables recogidas durante el embarazo										
Variables sociodemográficas, ambientales y dietéticas										
Área de estudio	Valencia, Gipuzkoa									
Estación toma de muestra	Primavera, verano, otoño, invierno									
Edad de la madre	Continua (años)									
Edad del padre	Continua (años)									
Nivel de estudios de la madre	Estudios primarios, secundarios, universitarios									
Nivel de estudios del padre	Estudios primarios, secundarios, universitarios									
País de nacimiento de la madre	España, otro país España, Latinoamérica, otro país									
IMC materno antes del embarazo	Continua (kg/m2) Categórica (<25, 25≤30, ≥30)									
Paridad	0, ≥1									
Empleo materno	Trabajadora, no trabajadora									
Empleo paterno	Trabajador, no trabajador									
Clase social parental	I+II (alta), III (media), IV+V (baja)									
Consumo de tabaco materno[1]	Sí, no									
Consumo de tabaco paterno[2]	Sí, no									
Consumo de alcohol[1] materno	Sí, no									
Área de residencia	Rural, no rural									
Proximidad de la residencia a un área industrial[2]	Sí, no									
		Artículo I			Artículo II			Artículo III		

		E	R	C	E	R	C	E	R	C
Covariables (cont.)										
Variables recogidas durante el embarazo (cont.)										
Variables sociodemográficas, ambientales y dietéticas (cont.)										
Proximidad de la residencia a un área agrícola[2]	Sí, no			■			■			■
Frecuencia del tráfico cerca de la residencia[2]	Continuamente, bastante frecuente, infrecuente/nunca			■			■			
Variables dietéticas[1]	Continua (Raciones/semana y gramos/día, ajustadas por calorías).			■			■			■
Estimación ingesta nutrientes[1]	Continua y categórica (unidades y categorías según el tipo de nutriente)						■			■
Ingesta de suplementos vitamínicos[1]	Sí, no			■			■			
Exposición a $PM_{2.5}$ [1]	Continua ($\mu g/m^3$)						■			
Biomarcadores recogidos durante el embarazo										
Selenio suero materno[1]	Continua (µg/L) Categórica (< o ≥ a la mediana)			■			■			■
Zinc orina materna[1]	Continua (µg/L) Categórica (< o ≥ a la mediana)									■
Ferritina suero materno[1]	≤15, > 15 mg/L			■			■			■
Cadmio orina materna[1]	Continua (µg/L)						■			■
Creatinina orina materna[1]	Continua (µg/L)						■			■
Variables recogidas en el nacimiento										
Edad gestacional	Continua (semanas)			■						■
Sexo del niño/a	Niño, niña			■						■
Variables recogidas al año										
Duración de la lactancia materna	0 semanas, >0-16 semanas, >16-24 semanas, >24 semanas			■						■
Empleo materno	Trabajadora, no trabajadora			■						
Empleo paterno	Trabajador, no trabajador			■						
Consumo de tabaco materno	Sí, no			■						
Consumo de tabaco paterno	Sí, no			■						
Principal cuidador	Madre, Madre y otros (padre, abuelos), otras combinaciones sin madre			■						
Asistencia a guardería	Sí, no			■						■
Variables recogidas los 4-5 años										
Empleo materno	Trabajadora, no trabajadora									■
Empleo paterno	Trabajador, no trabajador									■
Consumo de tabaco materno	Sí, no									■
Consumo de tabaco paterno	Sí, no									■
Principal cuidador	Madre, Madre y otros (padre, abuelos), otras combinaciones sin madre									■
Cociente de inteligencia verbal materna	Continua (puntuación de la escala WAIS-III)									■

Tabla 3. Variables de exposición, resultado y covariables utilizadas en los artículos incluidos en la tesis.

Nota: E: Exposición; R: resultado; C: covariable/confusor; As: arsénico; BSID: Escala Bayley de desarrollo infantil; MSCA: Escalas McCarthy de aptitudes y psicomotricidad para niños WAIS-III: escala de inteligencia para adultos de Wechsler, tercera edición. Cont.: continuación. [1]Información recogida durante el primer trimestre del embarazo; [2]Información recogida durante todo el embarazo

4.3 ANÁLISIS ESTADÍSTICO

4.3.1 Análisis descriptivo de los biomarcadores (artículos I, II, III)

Se calculó la media geométrica (MG) y los intervalos de confianza al 95% (IC95%) de las concentraciones de Mn y del As total y sus diferentes especies (DMA, MMA, iAs y AB), en total y según diferentes características sociodemográficas, clínicas, ambientales y dietéticas de la población. Las concentraciones de As fueron ajustadas por los niveles de creatinina con el fin de corregir la influencia de la dilución de las muestras de orina.

4.3.1.1 Calibración del arsénico (artículos II y III)

En estudios previos se ha utilizado la suma de las especies metiladas y no metiladas del As (DMA+MMA+iAs) como una aproximación de la exposición al iAs. Sin embargo, en poblaciones con alto consumo de pescado, los diferentes compuestos orgánicos de As (oAs) (como los arsenoazúcares y arsenolípidos) pueden contribuir a las concentraciones de DMA y TAs, por lo que podría no reflejar correctamente esta exposición (Navas-Acien et al., 2011). Para eliminar esta influencia, se ha utilizado el método matemático propuesto por Jones et.al., (2016). Este método utiliza las concentraciones de AB como marcador del consumo de pescado. Utilizando modelos de regresión lineal, se han estimado las concentraciones calibradas de iAs, DMA y MMA haciendo una regresión de las concentraciones de iAs, MMA y DMA en AB y las concentraciones de creatinina (todas las medidas se transformaron en logaritmo en base 2 [log2]) en tres modelos separados. Las nuevas concentraciones calibradas de iAs, MMA y DMA se han calculado sumando el residuo de cada modelo de metabolito a una constante (nivel medio de cada metabolito estimado a partir de participantes con AB <1 µg / L).

4.3.2 Evaluación de la eficiencia en la metilación del arsénico (artículos II y III)

Para evaluar la eficiencia en la metilación del As, que se ha usado como variable respuesta (**artículo II**) y como variable explicativa (**artículo III**), se han utilizado dos abordajes:

1) Calculando el porcentaje de las concentraciones calibradas de cada metabolito (DMA, MMA e iAs) sobre la suma de las tres especies (∑As).

2) Realizando un análisis de componentes principales (PCA) de los porcentajes de los metabolitos calibrados, no transformados. Los dos componentes obtenidos del análisis (componente principal 1 y 2 [PC1 y PC2], respectivamente]), los cuales explican el 100% de la varianza, han sido usados como fenotipos de metilación del As. La razón para realizar este análisis fue evitar la alta correlación entre los porcentajes de los tres metabolitos, transformando las tres variables interrelacionadas en dos medidas independientes.

4.3.3 Análisis de los factores asociados a las concentraciones de As y Mn y eficiencia en la metilación del arsénico (artículos I y II)

Para los siguientes análisis, se usaron los valores transformados mediante log2 de las concentraciones de especies de As en orina y Mn en suero y probit de los porcentajes de metabolitos, debido a que no seguían una distribución normal (evaluado tanto gráficamente como mediante el test Kolmogorov- Smirnov).

Se construyó un modelo de regresión lineal multivariante para cada una de las variables resultado (concentraciones de Mn, TAs, AB, ∑As, MMA, DMA, iAs, %DMA, %MMA, %iAs, PC1 y PC2) (ver Tabla 4). Se usó un procedimiento de dos en dos etapas: 1) se realizaron análisis bivariados entre las variables sociodemográficas y ambientales para cada variable resultado y se construyó un modelo con todas aquellas que obtuvieron un p valor<0,20. Mediante un proceso de eliminación hacia atrás, las variables con un p valor<0,10 en la prueba de razón de verosimilitud se mantuvieron en el modelo (modelo basal); 2) se incluyeron las variables dietéticas (grupos de alimentos, estimación y concentraciones de nutrientes) y otros compuestos (Cd en los modelos de metilación del As) en los modelos basales (modelos ajustados). Se retuvieron aquellas con un p valor <0,10 en la prueba de razón de verosimilitud.

	Variables resultado	Variables de exposición	
		Covariables (modelo basal)	Variables dietéticas y otros compuestos (modelo ajustado)
Modelo 1	Concentraciones de TAs, AB, ∑As, MMA, DMA Concentraciones de Mn	Covariables sociodemográficas y ambientales	Grupos de alimentos (expresados en continuo, raciones semanales)
Modelo 2	Concentraciones de TAs, AB, ∑As, MMA, DMA	Covariables sociodemográficas, y ambientales	Mismos grupos de alimentos que en modelo basal 1, pero consumo de pescado y carne expresados por subgrupos[1]
Modelo 3	%DMA, %MMA, %iAs, PC1 y PC2	Covariables sociodemográficas y ambientales	Estimación de nutrientes y concentraciones de Mn y Se sérico y Cd urinario

Tabla 4. Variables incluidas en cada modelo multivariante de regresión lineal (artículos 1 y 2).
[1]Los subgrupos utilizados para consumo de pescado fueron: pescado blanco, pescado azul y mariscos/moluscos; y para el consumo de carne: carne blanca y carne roja.

4.3.4 Análisis de la relación entre la exposición prenatal a Mn y As y el desarrollo neuropsicológico infantil (Artículos I y III)

Para los siguientes análisis, se usaron los valores transformados de log2 de las concentraciones de especies de As en orina y Mn en suero, y probit de los porcentajes de metabolitos, para corregir la asimetría de sus distribuciones.

Se construyeron los siguientes modelos de regresión lineal multivariante:

- Artículo I:
  - Variable exposición: concentraciones de Mn en suero materno.
  - Variable resultado: cada una de las escalas del test de neurodesarrollo utilizados al año (BSID).
- Artículo III:
  - Variable exposición: concentraciones de As total y sus metabolitos en orina materna, porcentajes de metabolitos de As, PC1 y PC2.
  - Variable resultado: cada una de las escalas del test de neurodesarrollo utilizados a los 4-5 años de edad (MSCA).

Se utilizó un procedimiento de dos etapas (ver **Tabla 5**): 1) se construyó un modelo basal para cada escala de los test con variables sociodemográficas, clínicas y ambientales como posibles covariables. Estos modelos se construyeron mediante un proceso de eliminación hacia atrás y utilizando los mismo criterios explicados en el punto anterior (ver **sección 4.3.3**); 2) se introdujeron las variables de exposición (log2 o probit transformadas) y se incluyeron potenciales factores de confusión si cambiaban la magnitud de los efectos principales de cada variable de exposición de manera significativa en comparación con el modelo ajustado por el mismo factor de confusión, pero aleatorizado (es decir, la misma variable reordenada aleatoriamente para simular independencia con las variables de exposición y respuesta), con un nivel de significación del 5% (P. H. Lee, 2014). El área de estudio (cohorte) y las concentraciones de creatinina (este último solo en los modelos de As) se incluyeron independientemente de su significatividad estadística.

Para evaluar la forma de la relación entre la exposición a los metales y las variables de desarrollo neurológico, se ajustaron modelos aditivos generalizados (GAM) y se compararon los resultados de los modelos lineales con los de los modelos no lineales mediante el criterio de información de Akaike (AIC) y la prueba de razón de verosimilitud (LRT).

La modificación del efecto se evaluó incluyendo el término de interacción en los modelos principales. Los modelos con y sin interacción se compararon con LRT y el efecto de la modificación se consideró estadísticamente significativo si el valor de p de la interacción fue <0,05.

Etapa 1.	Y variable de resultado $= \beta_0 + \beta_{2,3,4\ldots}$ covariables + $\beta_{9,10}$ variables forzadas + ε
Etapa 2.	Y variable de resultado $= \beta_0 + \beta_1$ variable de exposición $+ \beta_{2,3,4\ldots}$ covariables + $\beta_{5,6,7\ldots}$ factores de confusión $+ \beta_{9,10}$ variables forzadas $+ \varepsilon$
Variable resultado:	cada una de las subescalas de Bayley (artículo I) y MSCA (artículo III).
Variable de exposición:	concentraciones de Mn, TAs, AB, ∑As, MMA, DMA, %DMA, %MMA, %iAs, PC1 y PC2 (log2 o probit transformadas)
Covariables:	variables sociodemográficas, clínicas y ambientales con un p valor <0,10 en la prueba de razón de verosimilitud.
Factores de confusión:	variables sociodemográficas, clínicas, dietéticas y ambientales que cambiaban la magnitud de los efectos principales de cada variable de exposición de manera significativa en comparación con el mismo factor de confusión potencial.
Variables forzadas:	variables incluidas independientemente de su significatividad estadística.

Tabla 5. Proceso de construcción de los modelos multivariantes para el análisis de la relación entre la exposición prenatal a Mn y As y el desarrollo neuropsicológico infantil (artículos I y III).

4.3.5 Análisis de sensibilidad y diagnóstico de los modelos

Se realizaron diversos análisis de sensibilidad para evaluar la robustez de los modelos multivariables eliminando ciertos subgrupos de población, y utilizando como variables de exposición los porcentajes de metabolitos de As no calibrados en lugar de los calibrados, así como la inclusión de la variable de consumo materno de pescado en los modelos (artículo III).

En todos los modelos se comprobó si cumplían los supuestos de regresión lineal: 1) se inspeccionó gráficamente los residuos del modelo para verificar normalidad y homocedasticidad; 2) se identificaron datos influyentes mediante la distancia de Cook; 3) se comprobó la colinealidad entre las variables de los modelos mediante los factores de inflación de la varianza (VIF). Por último, se calcularon intervalos de confianza robustos en caso de observar pequeñas desviaciones respecto a la hipótesis de normalidad.

CAPÍTULO V. RESULTADOS

5.1 ARTÍCULO I

Soler-Blasco, R., Murcia, M., Lozano, M., González-Safont, L., Amorós, R., Ibarluzea, J., Broberg, K., Lopez-Espinosa, MJ., Lertxundi, N., Santa Marina, L., Ballester, F., Llop, S. (2020). Prenatal manganese exposure and neuropsychological development in early childhood in the INMA cohort. International Journal of Hygiene and Environmental Health, 224, 113443.

Disponible en: https://doi.org/10.1016/j.ijheh.2019.113443

Resumen

Introducción: El manganeso (Mn) es un elemento esencial, siendo la dieta la fuente principal. Algunos estudios epidemiológicos han encontrado que un exceso prenatal de Mn podría afectar negativamente el desarrollo neuropsicológico durante la infancia, pero la evidencia no es concluyente. El **objetivo** de este estudio fue explorar la relación entre las concentraciones de Mn en suero materno y el desarrollo neuropsicológico infantil evaluado al año de edad.

Métodos: Los sujetos de estudio fueron 1179 parejas madre-hijo de dos cohortes españolas (Valencia y Gipuzkoa) del Proyecto INMA (Medio Ambiente e Infancia). El Mn se midió en muestras de suero recogidas durante el primer trimestre del embarazo. El desarrollo neuropsicológico infantil se evaluó mediante las escalas de desarrollo infantil de Bayley, compuestas por la escala mental y psicomotora. La información sociodemográfica, de estilo de vida y dietética se recopiló mediante cuestionarios durante el embarazo y durante el primer año de vida. El Mn sérico se transformó en logaritmo base 2. Se construyeron modelos de regresión lineal multivariable. Se utilizaron modelos aditivos generalizados para evaluar la forma de la relación entre la exposición prenatal a Mn y las puntuaciones de las pruebas neuropsicológicas.

Resultados: la media geométrica y el intervalo de confianza al 95% (IC95%) del Mn en suero materno fue de 1,50 (1,48-1,53) µg/L. Los niveles de Mn fueron más altos entre las madres que no trabajan y en aquellas con un mayor consumo de frutos secos. La asociación entre los niveles de Mn materno y el desarrollo neuropsicológico infantil fue negativa en los modelos multivariables para las escala mental (β [IC del 95%] = −0,39 [−2,73 a 1,95]) y psicomotora (β [IC del 95%] = −0,92 [−3,48 a 1,65]), aunque los coeficientes no fueron estadísticamente significativos. La mejor forma que describe la relación entre Mn y las escalas de Bayley fue lineal en ambos casos. **Conclusión**: Este estudio no muestra asociación entre los niveles maternos prenatales de Mn y el desarrollo neuropsicológico al año del nacimiento en dos cohortes participantes en el estudio INMA.

International Journal of Hygiene and Environmental Health 224 (2020) 113443

Contents lists available at ScienceDirect

International Journal of Hygiene and Environmental Health

journal homepage: www.elsevier.com/locate/ijheh

Prenatal manganese exposure and neuropsychological development in early childhood in the INMA cohort

Raquel Soler-Blasco[a], Mario Murcia[a,b], Manuel Lozano[c,d], Llúcia González-Safont[a,b], Rubén Amorós[e], Jesús Ibarluzea[b,f], Karin Broberg[g], Amaia Irizar[h], Maria-José Lopez-Espinosa[a,b,i], Nerea Lertxundi[h,j], Loreto Santa Marina[b,f,h], Ferran Ballester[a,b,i], Sabrina Llop[a,b,*]

[a] Epidemiology and Environmental Health Joint Research Unit, FISABIO – Universitat Jaume I – Universitat de València, Valencia, Spain
[b] Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Madrid, Spain
[c] Foundation for the Promotion of Health and Biomedical Research in the Valencian Region, FISABIO-Public Health, Valencia, Spain
[d] Preventive Medicine and Public Health, Food Sciences, Toxicology and Forensic Medicine Department, Universitat de València, València, Spain
[e] School of Mathematics, University of Edinburgh, Edinburgh, United Kingdom
[f] Public Health Division of Gipuzkoa, Basque Government, San Sebastian, Spain
[g] Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden
[h] Biodonostia Health Research Institute, San Sebastian, Spain
[i] Department of Nursing, Universitat de València, Valencia, Spain
[j] Faculty of Psychology of the University of the Basque Country, San Sebastian, Spain

ARTICLE INFO

Keywords:
Manganese
Prenatal exposure
Neurodevelopment
Cognitive
Cohort studies
Spain

ABSTRACT

Introduction: Manganese (Mn) is an essential element, diet being its main source. Some epidemiological studies have found that a prenatal excess of Mn could negatively affect neuropsychological development during infancy, but the evidence is inconclusive. The aim of this study was to explore the relationship between maternal serum Mn concentrations and child neuropsychological development assessed at 1 year of age.

Methods: study subjects were 1179 mother–child pairs from two Spanish cohorts (Valencia and Gipuzkoa) of the INMA (Environment and Childhood) Project. Mn was measured in serum samples collected during the first trimester of pregnancy. Child neuropsychological development was assessed using the Bayley Scales of Infant Development, composed of both mental and psychomotor scales. Sociodemographic, lifestyle and dietary information was collected through questionnaires during pregnancy and during the child's first year of life. Serum Mn was log-2 transformed. Multivariable linear regression models were built. Generalized additive models were used to assess the shape of the relation between prenatal exposure to Mn and the neuropsychological test scores.

Results: geometric mean and 95% confidence interval (95% CI) of maternal serum Mn was 1.50 (1.48–1.53) μg/L. Levels of Mn were higher among non-working mothers and in those with a higher consumption of nuts. The association between maternal Mn levels and child neuropsychological development was negative in the multivariable models for the mental (β [95% CI] = −0.39 [-2.73, 1.95]) and psychomotor scales (β [95% CI] = −0.92 [-3.48, 1.65]), although the coefficients were not statistically significant. The best shape describing the relationship between Mn and the Bayley scales was linear in both cases.

Conclusion: This study shows a null association between maternal prenatal levels of Mn and neuropsychological development at one year after birth in two cohorts within the INMA study.

1. Introduction

Manganese (Mn) is an essential element involved in numerous metabolic processes, such as the metabolism of fats and carbohydrates, the formation of connective tissue and bones, and the synthesis and metabolism of neurotransmitters (Agency for Toxic Substances and Disease Registry, 2012; Santamaria and Sulsky, 2010). Mn is a naturally occurring metal in the environment. However, anthropogenic sources such as pesticides, gasoline additives and industry, especially metallurgical processes, have made a substantial contribution to the

* Corresponding author. Foundation for the Promotion of Health and Biomedical Research in the Valencian Region, FISABIO-Public Health, Avda. Catalunya 21, 46020, Valencia, Spain.
E-mail address: llop_sab@gva.es (S. Llop).

https://doi.org/10.1016/j.ijheh.2019.113443
Received 12 September 2019; Received in revised form 18 December 2019; Accepted 20 December 2019

Abbreviations

AIC	Akaike information criteria
BMI	body mass index
BSID	Bayley Scales of Infant Development
CI	confidence intervals
FFQ	food frequency questionnaire
GM	geometric mean
ICPMS	inductively coupled plasma mass spectrometry
ISCO88	International Standard Classification of Occupations coding system approved in 1988
IQR	interquartile range
LRT	Likelihood Ratio test
LOD	limit of detection
Mn	Manganese
n	sample size
NIST	National Institute of Standards and Technology
PM	particulate matter
ORS	octopole reaction system
SD	standard deviation

presence of Mn in the atmosphere (Lucchini et al., 2015).

The main exposure route in the non-exposed population is through diet. The main contributors to Mn levels are cereals, legumes, and vegetables (Agence nationale de sécurité sanitaire de l'alimentation de l'environnement et du travail, 2011; Filippini et al., 2017; Rose et al., 2010), although relatively high levels of Mn have been detected in other foods such as nuts, eggs, and tea (Perelló et al., 2015; Rose et al., 2010). The intake of water has been observed to be another source of exposure, especially in regions with high levels of Mn in drinking water, such as Bangladesh (Wasserman et al., 2006), Japan or Australia (Freeland-Graves et al., 2016). Likewise, living close to agricultural or industrial areas (Gunier et al., 2014; Takser et al., 2004) also seems to be related to Mn exposure.

Levels of Mn are kept within physiological limits by a homeostatic mechanism. An increase in Mn concentration can occur on account of exposure to high levels of Mn or owing to the presence of conditions that can affect this homeostatic mechanism, such as iron deficiency or liver dysfunction (Agency for Toxic Substances and Disease Registry, 2012). There is scarce evidence about the neurological effects caused by exposure to Mn in the general population or in critical periods, as in prenatal development (Agency for Toxic Substances and Disease Registry, 2012).

Epidemiological studies have reported an increase in blood Mn levels during pregnancy (Spencer, 1999; Takser et al., 2004). Mn is transferred from the mother to the fetus via the placenta through active transport mechanisms in order to meet the fetal demand for this element (Nandakumaran et al., 2016). Mn is able to cross the blood-brain barrier and accumulate in the developing brain (Yoon et al., 2011). Although Mn is an essential element for fetal growth, the developing brain seems to be especially vulnerable to an excess of Mn (Grandjean and Landrigan, 2014).

Very few epidemiological studies have evaluated the relationship between prenatal Mn levels and neuropsychological development in infancy. Takser et al. (2003) found a negative relation between cord blood Mn levels and hand skills, attention and non-verbal memory scores at 3 years of age, but no association was observed between Mn and general cognitive scores at 3 (n = 126) and 6 (n = 100) years old. Claus-Henn et al. (2017) reported a significant negative association between maternal blood Mn levels and mental and psychomotor development in 2-year-old children from USA (n = 224). Some studies have observed an inverted U-shaped relationship between prenatal Mn levels and psychomotor, mental and/or language development in infancy (Chung et al., 2015; Muñoz-Rocha et al., 2018). Conversely, a recent study (n = 355) has found no significant relation between maternal blood Mn levels and neuropsychological development at 1 year of age (Mora et al., 2018).

Although the literature suggests that exposure to Mn during the prenatal period could affect children's neurodevelopment, this evidence is still too scarce to draw any definite conclusions. Therefore, the aim of this study was to explore the relationship between maternal Mn concentrations and child neuropsychological development assessed at around 12 months of age in a Spanish birth cohort study. We also described the maternal exposure to Mn and assessed the influence of sociodemographic, environmental and dietary characteristics on it.

2. Material and methods

2.1. Study population

The study population belongs to the INMA Project (Environment and Childhood), a multicentre birth cohort study that aims to investigate the effect of environmental exposures and diet during pregnancy and childhood on fetal and child development in different geographical areas of Spain (http://www.proyectoinma.org). Subjects were participants in the Gipuzkoa (northwest of Spain) and Valencia (east of Spain) areas.

The study protocol has been reported elsewhere (Guxens et al., 2012). Briefly, 1493 pregnant women were recruited during their first antenatal visit (2003–2008, Gipuzkoa = 638, Valencia = 855). The inclusion criteria were: at least 16 years of age, 10–13 weeks of gestation, singleton pregnancy, intention of undergoing follow-up and delivery in the corresponding centre of reference, and no impediment for communication. Excluding the women who withdrew from the study, were lost to follow-up, and had induced or spontaneous abortions or fetal deaths, of the total sample we followed up 1399 (93.7%) women until delivery. Their children were enrolled at birth and were followed up until they were 1 year old. The final study population was made up of 1179 mother–child pairs in whom both manganese levels in maternal serum and neurodevelopmental assessment at age 1 were available (84.3% of total births).

Analyses were performed to determine any differences between the population included in the study and those who were not included (n = 220, [15.7% of total births]). Among the participants, mothers were slightly older, with a higher level of maternal education and higher social class than in the case of non-participants (Table S1).

Informed consent was obtained from all participants in each phase, and the study protocol was approved by the Ethics Committee of the University Hospital La Fe (Valencia), the Ethics Committee of the Public Health Research Centre in Valencia (CSISP) and the Ethics Committee of Donostia Hospital (Gipuzkoa).

2.2. Variables and sources of information

2.2.1. Manganese analysis

Concentrations of Mn were determined in serum samples taken at the first trimester of pregnancy (mean number of weeks of gestation 13.0 [standard deviation (SD): 1.9]). After separation of serum by centrifugation, samples were stored at −80 °C and transported frozen to the Karolinska Institutet, Sweden, for analysis. Serum samples were prepared for analysis by a direct alkali dilution method (Levi et al., 2018). Briefly, samples were diluted 1:15–50 with an alkali solution consisting of 2% butanol (Honeywell Research Chemicals, Seelze, Germany), 0.05% EDTA (Sigma-Aldrich, St. Louis MO, USA), 0.05% Triton X-100 (Sigma-Aldrich), 1% NH_4OH (Romil, Cambridge, UK) and 20 μg/g of the internal standards Sc, Ge and Rh. The concentrations of serum Mn were determined by inductively coupled plasma mass

spectrometry (ICPMS; Agilent 7700x, Agilent Technologies, Tokyo, Japan) equipped with an octopole reaction system (ORS) collision/reaction cell technology. The limit of detection (LOD) was determined as 3 x SD of analyzed blanks (alkali solution) and as signal/noise = 3. The accuracy and precision of each analysis was verified by analyzing commercially available reference materials: Seronorm human serum lot MI0181 (SERO, Billingstad, Norway) and NIST animal serum SRM 1598a (National Institute of Standards and Technology, Gaithersburg MD, USA). Blanks and reference materials were treated along with the collected serum samples and analyzed at the beginning, in the middle and at the end of each analysis. Variations in Mn concentrations related to the day of laboratory analysis were observed, so the levels were standardized according to this variable.

2.2.2. Neuropsychological development assessment

Neurodevelopment of the children was assessed at 1 year of age (mean [SD] = 13.3 [1.3], range = 11.4–19.5 months of age) by using the first edition of the Bayley Scales of Infant Development (BSID) (Bayley, 1977). The BSID are composed of the mental and the psychomotor scales. The mental scale consisted of 163 items that assessed age-appropriate mental development in cognitive areas such as performance ability, memory, and first verbal learning. The psychomotor scale consisted of 81 items assessing fine and gross psychomotor development.

All testing was done in the corresponding health care centre in the presence of the mother, by a total of six trained psychologists. To limit inter-observer variability, we applied a strict protocol, including training sessions where inter-observer differences were quantified and the use of three sets of quality controls (inter-observer reliability tests). The inter-rater reliability estimated by intra-class correlation was 0.90 for mental test scores, and 0.91 for psychomotor test scores. Raw scores were standardized for child's age in days at test administration and for psychologist. Standardized residuals were then typified by having a mean of 100 points (SD = 15) to homogenize the scales.

2.2.3. Other variables

Women filled in two questionnaires during their pregnancy, at the first and the third trimesters of gestation (mean [SD] number of weeks of gestation 13.21 [1.58] and 32.3 [2.21], respectively). The questionnaires were administered by trained interviewers and focused on socio-demographic, environmental and lifestyle information during pregnancy. The covariates used in this study were maternal and paternal age at conception (years), parental education level (up to primary, secondary, university), maternal country of birth (Spain, other), body mass index (BMI, kg/m^2) before pregnancy (low weight [< 18.5], healthy [18.5- < 25], overweight [25- < 30], obesity [≥30]), parity (0, ≥1), maternal working status at first trimester of pregnancy (non-working, working), maternal smoking habit until 12 weeks of gestation (smoker, non-smoker), and paternal smoking habit during pregnancy (smoker, non-smoker). Variables about the proximity of the residence to industrial or agricultural areas (yes, no) and frequency of traffic near the residence (continuous, rather frequent, infrequent/never) were also collected. We also obtained information about the maternal intake of multivitamin supplements until the 12th week of gestation (yes, no).

We defined parental social class from the maternal or paternal occupation during pregnancy with the highest social class, according to a widely used Spanish adaptation of the International Standard Classification of Occupations coding system approved in 1988 (ISCO88). Class I + II included managerial jobs, senior technical staff and commercial managers; Class III included skilled non-manual workers; and class IV + V included manual and unskilled workers.

Information on the children's gestational age and sex was obtained from clinical records. Information about the duration of breastfeeding (weeks), maternal and paternal working status at 1 year of age (non-worker, worker), maternal and paternal smoking habit (smoker, non-smoker), main care provider (mother, mother and others, other combinations without mother), and attendance at nursery (yes, no) was obtained at the same time as the neuropsychological assessment. Breastfeeding was defined as receiving breast milk, although this could be supplemented with any food or liquid, including non-human milk.

Information on diet during pregnancy was obtained from a semi-quantitative food frequency questionnaire (FFQ) administered at the first and third trimester. This FFQ was validated in the Valencia cohort with good reproducibility for nutrient and food intake (Vioque et al., 2013). Only the information derived from the first trimester FFQ was used for this study due to the temporal proximity to the Mn measurement. The items had nine possible responses, ranging from 'never or less than once per month' to 'six or more per day'. A commonly used serving size was specified for each food item in the FFQ. This was converted to average daily intake in grams for each individual participant. We obtained data (expressed in grams per week) on the intake of dairy products, eggs, meat, seafood, fruits, vegetables, legumes, nuts, potatoes, cereals and bread, and coffee and other infusions (such as tea). We also obtained information about consumption of tap water. Energy-adjusted intakes were computed using the residual method, where the residuals were calculated from a linear regression, with the natural logarithm of the food group modeled as the dependent variable and the natural logarithm of total energy intake as the independent variable.

The concentrations of serum selenium (Se) were determined by inductively coupled plasma mass spectrometry with the collision/reaction cell system in hydrogen mode. More information about the methodology used for Se analysis has been reported in detail elsewhere (Amorós et al., 2018).

Exposure to particulate matter with a diameter of less than 2.5 μm ($PM_{2.5}$) during pregnancy in the Gipuzkoa area was measured from the beginning of the study period until the last birth using three Digitel DHA-80 high-volume samplers. Descriptions of the study area, air pollution monitoring methodology, and particle quantification of the air quality in the area has been reported elsewhere (Lertxundi et al., 2015, 2010).

In Gipuzkoa the quantification of maternal plasmatic levels of ferritin was performed by fluoroimmunoassay (DELFIA Ferritin kit A069-101), in the Gipuzkoa Public Health Laboratory. In Valencia, the quantification was performed at La Fe Hospital through immunoturbidimetry in Beckman Coulter AU analyzers.

2.3. Statistical analysis

We calculated the geometric mean (GM) and 95% confidence intervals (95%CI) of the Mn concentrations according to the socio-demographic, environmental and dietary characteristics of the study population.

For further analyses, the variable of maternal serum Mn concentrations was log2-transformed due to its skewed distribution. The ANOVA F-test was applied to the logarithm in order to compare the geometric mean of Mn concentrations across categories of the socio-demographic and environmental characteristics.

Bivariable and multivariable linear regression models were built in order to study the relationship between the maternal serum Mn concentrations at the first trimester of pregnancy and the socio-demographic, environmental and dietary factors. Point estimates and 95%CI for the beta (β) coefficients were obtained. The multivariable model was built using all the covariates associated with a p-value < 0.2 in the bivariable analysis, except diet variables. Following a backward elimination procedure, all the covariates associated with Mn concentrations at a level of p < 0.1 in the likelihood ratio test were retained in the model (basal model). In this model we included all diet variables expressed in 100 g/day at the same time. Although food intake variables were mutually correlated, we found no collinearity problems among them.

In order to assess the relation between prenatal exposure to Mn and

the neuropsychological test scores, multivariable linear regression models were built separately through a two-step procedure for the mental and psychomotor scales. In the first step, a core model was built for each scale with parental and child sociodemographic variables as possible covariates. These models were built using a backward elimination procedure with the same criteria as for the multivariable model for the Mn concentrations. In the second step, the log2 Mn variable was introduced into these adjusted models and additional potential confounders were included if they changed the magnitude of the Mn main effects in a significant way compared to the same potential confounder, but randomized (that is, the same variable randomly reordered to simulate independence from Mn and the response variable), with a 5% significance level (Amorós et al., 2019). All covariates in the multivariable model for the Mn concentrations were considered as potential confounders. Area of study (Valencia or Gipuzkoa) was included in all models regardless of their statistical significance.

Table 1
Total Mn concentrations in maternal serum at 12 gestational weeks according to socio-demographic and environmental characteristics, INMA Project (Valencia and Gipuzkoa, Spain, 2004–2008).

	N[a] (%)	GM	95%CI		Bivariable β (95%CI)	P[b]
Area of study						
Gipuzkoa	534 (45)	1.51	1.48	1.54		
Valencia	645 (55)	1.50	1.46	1.53		
Maternal age (years)						
< 25	77 (7)	1.48	1.38	1.58		
25-29	382 (32)	1.50	1.47	1.54	0.02 (−0.08, 0.12)	0.65
30-34	527 (45)	1.51	1.47	1.55	0.03 (−0.07, 0.13)	0.56
≥35	193 (16)	1.49	1.44	1.55	0.01 (−0.10, 0.12)	0.83
Maternal country of birth						
Spain	1082 (92)	1.51	1.48	1.53		
Others	97 (8)	1.48	1.41	1.56	−0.02 (−0.10, 0,06)	0.63
BMI before pregnancy (Kg/m[b])						
Healthy (18.5– < 25)	844 (72)	1.51	1.42	1.60	0,01 -0,11 0,130.19	
Low weight (< 18.5)	45 (4)	1.49	1.47	1.52	0.01 (−0.11, 0.13)	0.85
Overweight (25– < 30)	207 (18)	1.56	1.50	1.62	0.06 (0.00, 0.12)	0.04
Obesity (> 30)	83 (7)	1.46	1.39	1.54	−0.03 (−0.12, 0.06)	0.56
Parity						
0	656 (56)	1.51	1.48	1.54		
≥1	523 (44)	1.50	1.46	1.53	−0.01 (−0.06, 0.04)	0.69
Parental social class						
I + II (high)	393 (33)	1.50	1.46	1.54		
III	293 (25)	1.49	1.44	1.54	−0.01 (−0.07, 0.05)	0.80
IV + V (low)	493 (42)	1.51	1.48	1.55	0.01 (−0.04, 0.07)	0.61
Maternal educational level[b]						
Up to primary	272 (23)	1.53	1.46	1.59		
Secondary	471 (40)	1.49	1.46	1.53	−0.03 (−0.09, 0.03)	0.26
University	434 (37)	1.50	1.47	1.54	−0.03 (−0.09, 0.04)	0.40
Maternal working status at 12 wg						
Non-working	261 (22)	1.54	1.49	1.60		
Working	916 (78)	1.49	1.47	1.52	−0.05 (−0.11, 0.06)	0.07
Proximity to agricultural area						
Yes	454 (39)	1.50	1.46	1.54		
No	712 (61)	1.51	1.48	1.53	0.01 (−0.04, 0.05)	0.83
Proximity to industrial area						
Yes	385 (33)	1.52	1.47	1.56		
No	781 (67)	1.50	1.47	1.47	−0.02 (−0.07,0.03)	0.48
Frequency of traffic near the residence home						
Continuous	420 (36)	1.50	1.46	1.54		
Rather frequent	275 (24)	1.53	1.48	1.59	0.03 (−0.03, 0.09)	0.30
Infrequent or never	470 (40)	1.49	1.45	1.52	−0.01 (−0.06, 0.04)	0.67
$PM_{2.5}$ exposure at 12wg[c]	18.0 (2.9)[d]	–	–	–	−0.01 (−0.02, 0.005)	0.26
Maternal smoking habit until 12 wg						
Non-smoker	942 (81)	1.49	1.47	1.52		
Smoker	223 (19)	1.53	1.47	1.60	0.04 (−0.02, 0.10)	0.18
Maternal ferritin serum at 12 wg						
≥15 mg/L	870 (81)	1.50	1.47	1.53		
< 15 mg/L	209 (19)	1.53	1.48	1.59	0.03 (−0.03,0.09)	0.34
Maternal selenium serum at 12wg	79.8 (9.5)[d]	–	–	–	0.004 (0.002, 0.007)	< 0.01
Tap water consumption						
Less than 1 glass (250 cc) at day	590(51)	1.49	1.46	1.53		
1 or more glass (250 cc) at day	596 (49)	1.52	1.49	1.55	0.02 (−0.04, 0.074)	0.52
Multivitamin supplementation until 12 wg						
No	986 (67)	1.52	1.49	1.55		
Yes	478 (33)	1.48	1.43	1.52	−0.05 (−0.11, 0.01)	0.12

[a] Missing values for some variables not included in percentages: maternal working status at 12 wg (2); paternal education level (8); Frequency of traffic near the residence (14); Proximity to agricultural area (13); Proximity to industrial area (13); Maternal smoking habit until 12 wg (14); Maternal ferritin serum at 12 wg (100); Multivitaminic supplementation until 12 wg (29).

[b] p-value from ANOVA F- test (bivariable analysis, adjusted for area of study [Gipuzkoa, Valencia]).

[c] Data only available for Gipuzkoa area (n = 468).

[d] Mean and standard deviation. N: sample size; BMI: Body mass index; SC: Social Class; GM: geometric mean; 95%CI: 95% confidence intervals; wg: weeks of gestation; PM: particulate matter.

Generalized additive models were fitted to evaluate non-linear patterns. Natural cubic splines with one or two internal knots were compared through Akaike information criteria (AIC). The lowest AIC non-linear model and linear model were then compared using graphical examination and the Likelihood Ratio test (LRT).

Effect modification by sex of the child, maternal serum Se, maternal serum ferritin, and maternal intake of multivitamin supplements were assessed by including the interaction in the main models. The models with and without interaction were compared with LRT and the effect of modification was considered statistically significant if the p-value < 0.05.

Several sensitivity analyses were performed to evaluate the robustness of the multivariable models and these were repeated after eliminating certain population subgroups: preterm birth (n = 48), low birth weight (n = 55), children with an underlying pathology (such as very preterm, epilepsy, hypotonia, plagiocephaly, or fetal suffering, n = 16), or those in whom the quality test was uncertain (n = 85). The final models were also repeated excluding an extreme outlier (studentized residuals > 8).

The validity of the regression models was tested by residual analyses: normality and homoscedasticity were verified graphically. No influential data were identified by Cook's distance. Collinearity diagnostics were conducted on the final models. To deal with the possibility of minor deviations from normality and homoscedasticity, confidence intervals were calculated on the basis of robust standard error in the final results.

Statistical analysis was carried out using the R statistical package version 3.5.1 (R Core Team, 2017).

3. Results

In our population, 45% of the mothers were 30–34 years old, around 92% were born in Spain, around 40% had a university degree, and more than the 40% belonged to the lowest social class (Table 1).

Mn concentrations were detected in all maternal serum samples. The GM of serum Mn concentration was 1.50 (95%CI: 1.48–1.53) μg/L, with an interquartile range (IQR) from 1.30 to 1.67 μg/L and a median of 1.45 μg/L. Higher Mn levels were observed among mothers born in Spain, those who lived near an industrial area or with anemia at the first trimester of pregnancy (serum ferritin lower than 15 μg/L), although the differences did not reach the significance level (see Table 1). Marginally significant higher Mn levels were observed among non-working mothers at the first trimester of pregnancy (GM [95%CI]: 1.49 [1.47, 1.52] μg/L for working mothers vs. 1.54 [1.49, 1.60] μg/L for non-working mothers, p-value = 0.04). Women who were overweight before pregnancy also had higher Mn concentrations than those with a healthy BMI (GM [95%CI]: 1.56 [1.50, 1.62] μg/L vs. 1.51 [1.42, 1.60] μg/L), the coefficient in the bivariable linear regression model being statistically significant (β [95%CI] = 0.06 [0.00, 0.12], p-value = 0.04).

The variables associated with the maternal Mn serum concentration at the first trimester of pregnancy in the multivariable model were maternal working status (β [95%CI] = −0.05 [-0.10, 0.00], p = 0.04) and consumption of nuts (β [95%CI] = 0.21 [0.01, 0.41], p = 0.04). The association with consumption of coffee and other infusions (such as tea) was marginally significant (β [95%CI] = 0.01[0.00, 0.02], p = 0.07). No other dietary variable was associated with Mn levels (Table 2).

The association between maternal Mn levels and neuropsychological development was negative in the multivariable models for both scales (β [95% CI] = −0.39 [-2.73, 1.95] for the mental scale; β [95% CI] = −0.92 [-3.48, 1.65]) for the psychomotor scale); nevertheless, the coefficients were not statistically significant (Table 3, Model 1). Subsequently, serum ferritin and serum Se levels during pregnancy were also included in the model (Table 3, Model 2 and Model 3), and similar results were obtained.

Non-linear patterns in the relation between serum Mn and each Bayley subscale were evaluated. In both models, the non-linearity of multivariable GAM did not improve the model's fit (p > 0.05). Fig. 1 showed the linear relationship between prenatal Mn levels and the mental and psychomotor scales. None of the variables considered as potential effect modifiers (sex of the newborn, ferritin levels at first trimester of pregnancy, maternal serum Se at first trimester of pregnancy and maternal intake of multivitamin supplements) produced a significant change in the association between Mn exposure and the child's mental and psychomotor scales (results for sex and ferritin in supplemental material Table S2 and Figure S1). No significant changes were found in the results when sensitivity analyses were performed (supplemental material Table S3).

4. Discussion

In this Spanish birth cohort study, a null association was found between maternal serum Mn and mental or psychomotor development at year of age. As far as we know, this is the largest epidemiological study to have analyzed the association between prenatal Mn and neuropsychological development in early childhood.

Evidence about prenatal Mn exposure and neuropsychological development is still inconclusive. In fact, a recent systematic review concluded that the available evidence does not demonstrate adverse effects of early-life Mn exposure on neuropsychological development, and more prospective studies are needed to establish a causal relation (Leonhard et al., 2019).

Table 4 shows the prospective studies that have evaluated this relation using Mn measured in maternal or cord blood as a biomarker of prenatal exposure. In agreement with our results, a recent study carried out in Costa Rica on a population with high exposure to Mn through pesticides did not find an association between maternal blood Mn (median = 24.0 μg/L) and any scale of BSIDIII assessed at 1 year of age (Mora et al., 2018). In the same way, Takser et al. (2003) found no relationship between Mn, measured in maternal blood at delivery (GM = 20.4 μg/L), and children's neuropsychological development assessed at 6 months and 3 and 6 years of age. Nevertheless, the same authors observed a statistically significant negative relationship between cord blood Mn (geometric mean = 38.5 μg/L) and attention, non-verbal memory and hand skills assessed at 3 years old. Conversely, Claus-Henn et al. (2017) observed a statistically significant inverse association between Mn measured in maternal blood

Table 2
Multivariate linear regression between maternal serum Mn and socio-demographic and dietary factors. INMA Project (Valencia and Gipuzkoa, Spain, 2004–2008).

	Beta	95%CI		p-val[a]
Maternal working status				
Non-working				
Working	−0.05	−0.10	0.00	0.04
Dairy products [b]	0.00	−0.01	0.01	0.70
Eggs[b]	−0.07	−0.33	0.19	0.59
Meat[b]	0.01	−0.03	0.06	0.63
Seafood and shellfish[b]	−0.01	−0.07	0.06	0.86
Fruits[b]	0.00	−0.01	0.01	0.91
Vegetables[b]	−0.01	−0.03	0.02	0.64
Nuts[b]	0.21	0.01	0.41	0.04
Legumes[b]	−0.05	−0.15	0.04	0.30
Potatoes[b]	−0.03	−0.09	0.04	0.40
Cereals and bread[b]	−0.01	−0.05	0.02	0.45
Coffee and others infusions[b]	0.01	0.00	0.02	0.07

95%CI: 95% confidence intervals.
Model adjusted for area of study and all variables in the table.
[a] p-value from ANOVA F- test (adjusted for area of study [Gipuzkoa, Valencia]).
[b] 100 g per day at first trimester of pregnancy, adjusted by calories.

Table 3
Multivariate linear regression analysis between maternal Mn levels and child neuropsychological development assessed by Bayley Scores at 1 year old. INMA Project, Spain.

	Mental scale				Psychomotor scale			
	beta	95%CI		P[a]	beta	95%CI		P[a]
Model 1	−0.39	−2.73	1.95	0.74	−0.92	−3.48	1.65	0.43
Model 2	−0.41	−2.83	2.01	0.74	−0.96	−3.72	1.80	0.43
Model 3	−0.30	−2.64	2.04	0.80	−0.77	−3.35	1.82	0.56

Maternal serum Mn concentrations were log2 transformed.
Model 1: All models adjusted for children's age at evaluation, psychologist, area of study (Gipuzkoa/Valencia), child's sex and consumption of nuts at first trimester of pregnancy.
Mental scale model additionally adjusted for maternal age and body mass index before pregnancy.
Psychomotor scale model additionally adjusted for parental social class, maternal educational level and paternal age.
Model 2: model 1 + maternal serum ferritin (log2 transformed) at first trimester of pregnancy.
Model 3: model 1 + maternal serum selenium at first trimester of pregnancy.
[a] p-value from ANOVA F-test.

(median = 24.0 μg/L), but not in cord blood (median = 43.1 μg/L), and the mental and psychomotor scales assessed using the BSIDII at 24 months of age.

In our study, the non-linear models did not improve the model's fit. These results are in agreement with those from Mora et al. (2018), who found that the shape of most associations between maternal blood Mn during pregnancy and neurodevelopment assessed through BSIDIII scale at 1 year of age was linear. Muñoz-Rocha et al. (2018) also evaluated the shape of the relationship between Mn levels, measured in maternal and cord blood, and child neuropsychological development assessed in 2-year-old children from Mexico. They observed non-linear patterns for the cord blood Mn levels (mean = 50.1 μg/L) but linear ones for the maternal levels (mean = 27.7 μg/L). Some studies have suggested the existence of a threshold value in Mn levels above which the association with neuropsychological development becomes evident. Thus, a Korean cohort reported an inverse association between prenatal Mn and the mental and psychomotor development at 6 months old only when maternal blood Mn levels were > 30 μg/L (Chung et al., 2015). Similarly, Lin et al. (2013) found a negative association between cord blood Mn levels and cognitive and language scores at levels above 59 μg/L in 2-year-old children from Taiwan. This disparity between the results of our study and findings in other works may be due to the fact that Mn concentrations in our population (GM [95%CI] = 1.50 [1.48–1.53] μg/L in maternal serum) could be within a normal/homeostatic range, in which this compound behaves as an essential element.

The literature on the relationship between prenatal Mn levels and neuropsychological development in childhood is still scarce and the results observed are too heterogeneous to draw any definite conclusions. The inconsistency in the findings could be due to the use of different matrices to measure Mn concentrations (whole blood, serum and plasma). After absorption, Mn is rapidly distributed to the organs through the blood. The liver is the main organ that regulates Mn levels in the body. Excess Mn is sequestered by the liver and excreted into the

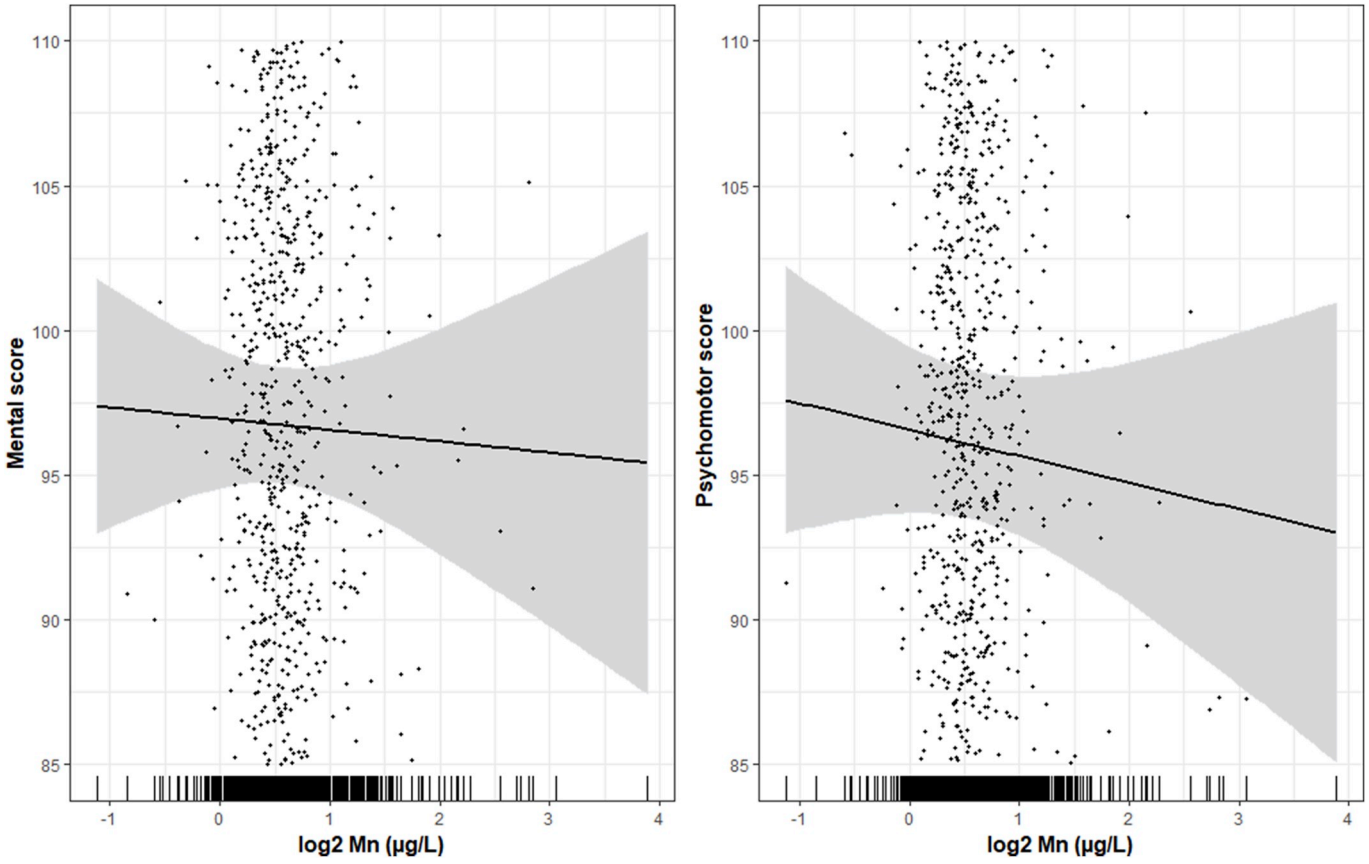


Fig. 1. Association between maternal serum Mn at first trimester of pregnancy and the mental and psychomotor scales assessed by the Bayley Scores of Infant Development at 12 months of age. INMA Project (Valencia and Gipuzkoa, Spain).
Both models adjusted for children's age at evaluation, psychologist, area of study child sex and nuts consumption at first trimester of pregnancy. Mental scale model additionally adjusted for maternal age and body mass index before pregnancy. Psychomotor scale model additionally adjusted for parental social class, maternal educational level and paternal age.

Table 4
Longitudinal studies on prenatal exposure to Mn (measured on maternal serum or cord blood) and Children's neuropsychological development.

Study	Country	N pairs	Mn matrix	Gestation age	Prenatal exposure levels [median (P25,75)] μg/L	Age of children at test	Neuro-development test/scales		Effect
Present study	Spain	1179	Maternal serum	First trimester	1.45 (1.30, 1.67)	1yr	*BSID* ● Mental ● Psychomotor		Ns for any scale
Mora et al. (2018)	Costa Rica	355	Maternal whole blood	First, second or third trimester	24.0 (20.3, 28)	1yr	*BSID-III* ● Cognition ● Motor function	● Language ● Social-emotional	Ns for any scale
Muñoz-Rocha et al. (2018)	Mexico	473	Maternal blood	Third trimester (between 30 and 34 weeks)	27.7 (8.7) [a]	24mo	*BSID-III* ● Cognition ● Motor function ● Language		– for cognitive scale – for motor scale – for language scale
Claus Henn et al. (2017)	USA	224	Maternal blood	At birth (± 12h)	24.0 (19.5, 29.7)	24mo	*BSID-II* ● Mental ● Psychomotor		– for mental development – for psychomotor development
			Cord blood		43.1 (33.5, 52.1)				Ns for any scale
Chung et al. (2015)	Korea	232	Maternal whole blood	Before delivery	22.5 (6.5)[a]	6mo	*BSID-II* ● Mental ● Psychomotor		– for mental development (change from positive to negative association in levels above 24–26 μg/L). – for psychomotor development (change from positive to negative association in levels above 26–28 μg/L).
Yu et al., 2014	China	933	Cord serum		4 .1 (2.7, 9.0)	3 days after birth	*NBAS* ● Total score ● Behavior ● Active and passive tone	● Primary reflexes ● General reactions	– for total score, active tone, behavior and general reactions, from levels above 5 μg/L Ns for passive tone and primary reflexes
Lin et al. (2013)	Taiwan	230	Cord blood		47.9 (39.7, 59.3)	2yrs	*CDIIT* ● Whole test ● Cognitive ● Language ● Motor	● Gross and fine motor ● Social ● Self-help	– cognitive and language development Ns for whole test, motor, gross and fine motor, social and self-help
Takser et al. (2003)	France	195, 126, 100	Maternal blood	At delivery	20.4 (11.1, 40.4)[b]	6mo 3yrs 6yrs	*Brunet–Lézine scales* ● Developmental quotient	*MSCA* ● General Cognitive Index ● Non-verbal memory ● Attention ● Hand-skill	Ns any neurodevelopment test at any age
			Cord blood		38.5 (19.1–71.2)[b]				– non-verbal memory at 3yrs – attention at 3yrs – hand skill at 3yrs, only in boys

ADHD: Attention deficit hyperactivity disorder; BSID-II: Bayley Scales of Infant and Toddler Development, 2nd edition; BSID-III: Bayley Scales of Infant and Toddler Development, 3rd edition; CDIIT: Comprehensive Developmental Inventory for Infants and Toddlers; NBAS: Neonatal Behavioral Assessment Scale; MSCA: McCarthy Scales of Children's Abilities.
Ns: not significant; −: inverse and significant association; mo: months old; yrs: years old. N: sample size; Mn: Manganese.
[a] Mean (standard deviation).
[b] Geometric mean (p5, p95).

bile, maintaining an adequate physiological level of Mn in the plasma. For this reason, Mn has a short half-life in blood and plasma/serum, resulting in a weak relationship between indicators of Mn exposure and Mn status (EFSA Panel on Dietetic Products Nutrition and Allergies, 2013; Zheng et al., 2011). However, Mn serum concentrations seem to be slightly sensitive to large variations in Mn intake, but this fact is not conclusive (Institute of Medicine (US) Panel on Institute of Medicine (US) Panel on Micronutrients, 2001). In 2013, the European Food Safety Authority concluded that, due to the efficient homeostatic mechanism, there was no reliable biomarker of Mn status, thus resulting in a weak relationship between indicators of Mn exposure and Mn status (EFSA Panel on Dietetic Products Nutrition and Allergies, 2013; Lucchini et al., 2015).

Differences between the present study and previous ones as regards sample collection could also be influencing the heterogeneity of the results. In most of the previous studies, Mn concentrations were measured in the final period of pregnancy or at birth. It has been observed that there is a tendency toward increased maternal Mn levels during pregnancy (Spencer, 1999; Takser et al., 2004), probably related to a higher intestinal absorption of this element due to the physiological iron deficiency during this period (Abbassi-Ghanavati et al., 2009; Finley, 1999). The concentrations in cord blood have been observed to be consistently higher than in maternal blood (Arbuckle et al., 2016; Krachler et al., 1999; Takser et al., 2004), probably due to an active transport through the placenta (Krachler et al., 1999) or a more deficient homeostatic mechanism in fetuses than in adults (Aschner and Aschner, 2005). This fact and the medium-weak correlation between maternal Mn blood and cord blood Mn concentrations observed in several studies (Guan et al., 2013; Krachler et al., 1999; Zota et al., 2008) could be suggesting that cord blood Mn concentrations would be a better biomarker of prenatal exposure to Mn than maternal blood, but only for the last period of pregnancy due to the short half-life in blood (Agency for Toxic Substances and Disease Registry, 2012; Zheng et al., 2011).

In our population, the median of maternal Mn at the first trimester of pregnancy was 1.45 μg/l (IQR = 1.30–1.67) μg/L. These levels were lower than in other populations of pregnant women previously studied. For example, in a study conducted in China (Yu et al., 2013), the median of serum Mn levels observed was 2.8 μg/L. Similar levels were observed in a case-control study in Sweden, where the median of Mn in pregnant women from the control group was 2.7 μg/L (Ode et al., 2015). These differences could be explained, in part, by the differences in the period of sampling. In these two studies, the samples were collected at delivery, whereas in our study samples were collected at the first trimester of pregnancy.

Exposure to Mn through drinking water in the Spanish general population is low (approximately 99% of water supply controls were below the WHO guide value of 400 μg/L) (Palau Miguel et al., 2008). In addition, the daily intake of Mn estimated in some studies is situated within the range of the adequate daily requirement proposed by the WHO (2–5 mg per day) (Goñi and Hern, 2019; Rubio et al., 2009; World Health Organization, 1996). With regard to diet, in our study, consumption of nuts was the only factor associated with maternal Mn levels, even when the model was adjusted for the rest of the dietary variables. This type of food usually has high levels of Mn, reaching a concentration in some studies of around 12 mg/kg in France (Agence nationale de sécurité sanitaire de l'alimentation de l'environnement et du travail, 2011), 18 mg/kg in Italy (Filippini et al., 2018) or 25 mg/kg in the United Kingdom (Rose et al., 2010). Consumption of coffee and other infusions (such as tea) was marginally associated with maternal levels in our study. Tea in fact contains high levels of Mn (Agency for Toxic Substances and Disease Registry, 2012). In Rose et al. (2010), the beverage group (including tea, coffee, cocoa, chocolate and malt drinks) was the highest contributor of Mn to the population's dietary exposure (41%). Some total diet studies have shown that the main contributor to Mn levels in the general population was cereals, mainly due to the high frequency of consumption of these products (Filippini et al., 2018; Gimou et al., 2014; Rose et al., 2010; Rubio et al., 2009). Filippini et al. (2017) found the consumption of vegetables and legumes to be correlated with serum Mn in adults. Lastly, Irizar et al. (2019) found a significantly positive association between the consumption of eggs, cereals and nuts during pregnancy and neonatal hair Mn levels. Maternal working status was associated with Mn levels in our population, non-working mothers being the ones with the highest Mn concentrations. This subgroup had higher cereal consumption than working mothers (107 vs. 96.6 g/day, $p < 0.01$), but lower nut consumption 5.1 vs. 7.1 g/day, $p < 0.01$). Although in our study cereals were not related to Mn levels, this variable could reflect part of another unmeasured explanatory factor.

In the present study, women with iron deficiency (indicated through serum ferritin below 15 μg/L) presented slightly higher Mn levels than women with normal iron status (GM: 1.53 and 1.50 μg/L, respectively), although the differences were not statistically significant (p value = 0.34). There is experimental evidence of the competition between Mn and Fe in the blood brain barrier and, in fact, it has been suggested that both elements share absorption and transport mechanisms (Agency for Toxic Substances and Disease Registry, 2012). However, very few epidemiological studies have evaluated the relationship between iron and manganese. To the best of our knowledge, only one birth cohort study has evaluated the effect of the interaction between maternal iron status and prenatal Mn on child neurodevelopment, the results showing an inverse relationship between prenatal Mn levels and both mental and psychomotor development only among 6-month-old children whose mothers had lower haemoglobin concentrations. Nevertheless, the interaction was only significant for girls (Gunier et al., 2015). In our study, the interaction term between maternal serum Mn and serum ferritin was non-significant for any scales ($p = 0.82$).

Finally, in our study, we did not find an interaction between Mn exposure and child's sex (p interaction = 0.81 and 0.39 for mental and psychomotor scores, respectively). Previous evidence has shown that sex could modify the association between Mn exposure and neuropsychological development. Gunier et al. (2015) found a significant interaction between postnatal Mn exposure, measured in teeth dentine, and mental and psychomotor scales at 6 months. For prenatal exposure, the same study reported a significant interaction between prenatal Mn exposure and maternal haemoglobin levels for mental scale only in girls. Bauer et al. (2017) reported a U-shaped relationship between prenatal Mn levels measured in teeth dentine and visuospatial development, only in girls. In a recent study, Broberg et al. (2019) found a significant positive relationship between soil Mn levels and some neurobehavioral problems only in girls. Moreover, the same study demonstrated a genetic influence of the polymorphisms in genes coding Mn transporters SCL30A10 and SLC39A8, this influence being stronger in girls. In contrast, other studies did not find any effect modification of sex on postnatal Mn exposure and neurobehavioral functions (Bouchard et al., 2007; Oulhote et al., 2014).

This study has several limitations. Firstly, around 85% of the children recruited in the birth cohort participated in this study. This loss to follow-up could represent a bias in the estimation of some exposure-outcome associations, since the participants in the study had a more privileged socioeconomic profile than non-participants. Another drawback in our study could be related to the assessment of Mn exposure because the analysis of Mn has been carried out at only one time point during pregnancy. This could reflect only recent Mn exposure and hamper the evaluation of long-term exposures. As the vulnerability of the central nervous system extends from the beginning of pregnancy to adolescence, it would have been more appropriate to measure Mn at several time points during pregnancy, birth and the postnatal period. Infants are exposed to Mn mainly from their diet. As the Mn concentrations in breast milk are low (EFSA Panel on Dietetic Products Nutrition and Allergies, 2013), formula milk seems to be the main contributor to infants' Mn body burden (Frisbie et al., 2019). Thus, both

prenatal and postnatal exposure to Mn could be affecting children's neuropsychological development. Unfortunately, in the present study we have not assessed the postnatal exposure to Mn. Another limitation is that the maternal serum ferritin variable had 8.48% of missing data, which can lead to a loss of statistical power in the model where this variable was included. Finally, the present study has a lack of genetic information on Mn transporters, so the influence of this variable could not be proved. The major strength of our study is its prospective design, which made it possible to obtain sufficient and comprehensive information about maternal and children's characteristics, including levels of other metals and essential elements that may affect both Mn exposure and neuropsychological development. Another advantage would be the considerably large sample size in comparison with other previous studies.

5. Conclusion

In conclusion, the results of the present study did not suggest any effect of the early prenatal levels of Mn on neuropsychological development during the first year after birth. It is possible that the prenatal Mn levels observed may be within physiological limits, below the cut-off point from which this element could cause neurological adverse effects. These findings add information to the body of scientific knowledge about the adequate prenatal Mn levels and the window of susceptibility to its exposure. Nevertheless, similar epidemiological studies are necessary in order to improve our knowledge about the safe range of this compound in the prenatal period and the factors that can affect the homeostatic mechanism.

Declaration of competing interest

The authors declared no conflict of interest.

The study protocol was approved by the Ethics Committee of the university hospital La Fe (Valencia), the Ethics Committee of the Public Health Research Centre in Valencia (CSISP) and the Ethics Committee of Donostia Hospital (Gipuzkoa).

All study participants were included in the study after being signed the informed consent form.

Acknowledgments and funding

We are grateful to all the participants and their families for taking part in this study. We also appreciate the generous contribution in the study of all INMA members. A full roster of the INMA Project Investigators can be found at http://www.proyectoinma.org/presentacion-inma/listado-investigadores/en_listado-investigadores.html. This study was supported by grants from Instituto de Salud Carlos III [FIS-FEDER: 06/0867, 09/00090, 13/22187, 13/1944, 16/1288, 17/00663 and 19/1338; Miguel Servet- FEDER: CP11/0178 and CP15/0025; FIS-FSE: 17/00260; Miguel Servet-FSE: MS11/0178, MS15/0025, MSII16/0051], the Alicia Koplowitz Foundation 2017, and the Basque Government 2015111065, all of them from Spain.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ijheh.2019.113443.

References

Abbassi-Ghanavati, M., Greer, L.G., Cunningham, G., 2009. A reference table for clinicians. Obstet. Gynecol. 114, 1326–1331.

Agence nationale de sécurité sanitaire de l'alimentation de l'environnement et du travail, 2011. Second French Total Diet Study. ANSES Opinion.

Agency for Toxic Substances and Disease Registry, 2012. Toxicological Profile for Manganese. Agency for Toxic Substances and Disease Registry, Georgia.

Amorós, R., Murcia, M., González, L., Rebagliato, M., Iñiguez, C., Lopez-espinosa, M., Vioque, J., Broberg, K., Ballester, F., 2018. Maternal selenium status and neuropsychological development in Spanish preschool children. Environ. Res. 166, 215–222. https://doi.org/10.1016/j.envres.2018.06.002.

Amorós, R., Murcia, M., González, L., Soler-Blasco, R., Rebagliato, M., Iñiguez, C., Carrasco, P., Vioque, J., Broberg, K., Levi, M., Lopez-espinosa, M., Ballester, F., Llop, S., 2019. Maternal copper status and neuropsychological development in infants and preschool children. Int. J. Hyg Environ. Health 222, 503–512. https://doi.org/10.1016/j.ijheh.2019.01.007.

Arbuckle, T.E., Lei, C., Morisset, A., Fisher, M., Weiler, H., Mihai, C., Legrand, M., Davis, K., Ettinger, A.S., Fraser, W.D., Group, S., 2016. Maternal and fetal exposure to cadmium , lead , manganese and mercury : the MIREC study. Chemosphere 163, 270–282. https://doi.org/10.1016/j.chemosphere.2016.08.023.

Aschner, J.L., Aschner, M., 2005. Nutritional aspects of manganese homeostasis. Mol. Asp. Med. 26, 353–362. https://doi.org/10.1016/J.MAM.2005.07.003.

Bauer, J.A., Claus Henn, B., Austin, C., Zoni, S., Fedrighi, C., Cagna, G., Placidi, D., White, R.F., Yang, Q., Coull, B.A., Smith, D., Lucchini, R.G., Wright, R.O., Arora, M., 2017. Manganese in teeth and neurobehavior: sex-specific windows of susceptibility. Environ. Int. 108, 299–308. https://doi.org/10.1016/j.envint.2017.08.013.

Bayley, N., 1977. Escalas Bayley de Desarrollo Infantil. TEA Ediciones, Madrid.

Bouchard, M., Laforest, F., Vandelac, L., Bellinger, D., Mergler, D., 2007. Hair manganese and hyperactive behaviors: pilot study of school-age children exposed through tap water. Environ. Health Perspect. 115, 122–127. https://doi.org/10.1289/ehp.9504.

Broberg, K., Taj, T., Guazzetti, S., Peli, M., Cagna, G., Pineda, D., Placidi, D., Wright, R.O., Smith, D.R., Lucchini, R.G., Wahlberg, K., 2019. Manganese transporter genetics and sex modify the association between environmental manganese exposure and neurobehavioral outcomes in children. Environ. Int. 130. https://doi.org/10.1016/j.envint.2019.104908.

Chung, S.E., Cheong, H.K., Ha, E.H., Kim, B.N., Ha, M., Kim, Y., Hong, Y.C., Park, H., Oh, S.Y., 2015. Maternal blood manganese and early neurodevelopment: the mothers and children's environmental health (MOCEH) study. Environ. Health Perspect. 123, 717–722. https://doi.org/10.1289/ehp.1307865.

Claus Henn, B., Bellinger, D.C., Hopkins, M.R., Coull, B.A., Ettinger, A.S., Jim, R., Hatley, E., Christiani, D.C., Wright, R.O., 2017. Maternal and cord blood manganese concentrations and early childhood neurodevelopment among residents near a mining-impacted superfund site. Environ. Health Perspect. 125, 1–9. https://doi.org/10.1289/EHP925.

EFSA Panel on Dietetic Products Nutrition and Allergies, 2013. Scientific opinion on dietary reference values for manganese. EFSA J 11, 1–44. https://doi.org/10.2903/j.efsa.2013.3419.

Filippini, T., Cilloni, S., Malavolti, M., Violi, F., Malagoli, C., Tesauro, M., Bottecchi, I., Ferrari, A., Vescovi, L., Vinceti, M., 2018. Dietary intake of cadmium, chromium, copper, manganese, selenium and zinc in a Northern Italy community. J. Trace Elem. Med. Biol. 0– 1. https://doi.org/10.1016/j.jtemb.2018.03.001.

Filippini, T., Michalke, B., Grill, P., Malagoli, C., Malavolti, M., Vescovi, L., Sieri, S., Krogh, V., Cherubini, A., Maffeis, G., Lucchini, R., Ferrante, M., Vinceti, M., 2017. Determinants of serum manganese levels in an Italian population. Mol. Med. Rep. 15, 3340–3349. https://doi.org/10.3892/mmr.2017.6379.

Finley, J.W., 1999. Manganese absorption and retention by young women is associated with serum ferritin concentration. Am. J. Clin. Nutr. 70, 37–43.

Freeland-Graves, J.H., Mousa, T.Y., Kim, S., 2016. International variability in diet and requirements of manganese : causes and consequences. J. Trace Elem. Med. Biol. 38, 24–32. https://doi.org/10.1016/j.jtemb.2016.05.004.

Frisbie, S.H., Mitchell, E.J., Roudeau, S., Domart, F., Carmona, A., Ortega, R., 2019. Manganese levels in infant formula and young child nutritional beverages in the United States and France : comparison to breast milk and regulations. PLoS One 1–23. https://doi.org/10.1371/journal.pone.0223636.

Gimou, M.-M., Pouillot, R., Charrondiere, U.R., Noël, L., Guérin, T., Leblanc, J.-C., 2014. Dietary exposure and health risk assessment for 14 toxic and essential trace elements in Yaoundé: the Cameroonian total diet study. Food Addit. Contam. A 31, 1064–1080. https://doi.org/10.1080/19440049.2014.909953.

Goñi, I., Hern, A., 2019. Intake of nutrient and non-nutrient dietary antioxidants. Contribution of macromolecular antioxidant polyphenols in an elderly mediterranean population. Nutrients 11. https://doi.org/10.3390/nu11092165.

Grandjean, P., Landrigan, P.J., 2014. Neurobehavioural effects of developmental toxicity. Lancet Neurol. 13, 330–338. https://doi.org/10.1016/S1474-4422(13)70278-3.

Guan, H., Wang, M., Li, X., Piao, F., Li, Q., Xu, L., Kitamura, F., Yokoyama, K., 2013. Manganese concentrations in maternal and umbilical cord blood: related to birth size and environmental factors. Eur. J. Public Health 24, 150–157. https://doi.org/10.1093/eurpub/ckt033.

Gunier, R.B., Arora, M., Jerrett, M., Bradman, A., G, H.K., Mora, A.M., Kogut, K., Hubbard, A., Austin, C., Holland, N., Eskenazi, B., 2015. Manganese in teeth and neurodevelopment in young Mexican- American children. Environ. Res. 142, 688–695. https://doi.org/10.1111/ajt.13087.TIM-1.

Gunier, R.B., Mora, A.M., Smith, D., Arora, M., Austin, C., Eskenazi, B., Bradman, A., 2014. Biomarkers of manganese exposure in pregnant women and children living in an agricultural community in California. Environ. Sci. Technol. 48, 14695–14702. https://doi.org/10.1021/es503866a.

Guxens, M., Ballester, F., Espada, M., Ferna, M.F., Grimalt, J.O., Ibarluzea, J., Olea, N., Rebagliato, M., Tardón, A., Torrent, M., Vioque, J., Vrijheis, M., Sunyer, J., INMA project, 2012. Cohort Profile : the INMA — INfancia y medio ambiente —(environment and childhood) Project. Int. J. Epidemiol. 41, 930–940. https://doi.org/10.1093/ije/dyr054.

Institute of Medicine (US) Panel on Institute of Medicine (US) Panel on Micronutrients, 2001. Institute of Medicine (US) Panel on Micronutrients. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. National Academies

Press (US), Washington, DC.
Irizar, A., Gil, F., Lertxundi, A., Martín-domingo, M.C., Urbieta, N., Molinuevo, A., 2019. Manganese levels in newborns ' hair by maternal sociodemographic , dietary and environmental factors. Environ. Res. 170, 92–100. https://doi.org/10.1016/j.envres.2018.11.038.
Krachler, M., Rossipal, E., Micetic-Turk, D., 1999. Trace element transfer from the mother to the newborn - investigations on triplets of colostrum, maternal and umbilical cord sera. Eur. J. Clin. Nutr. 53, 486–494. https://doi.org/10.1038/sj.ejcn.1600781.
Leonhard, M.J., Chang, E.T., Loccisano, A.E., Garry, M.R., 2019. A systematic literature review of epidemiologic studies of developmental manganese exposure and neurodevelopmental outcomes. Toxicology 420, 46–65. https://doi.org/10.1016/j.tox.2019.03.004.
Lertxundi, A., Baccini, M., Lertxundi, N., Fano, E., Aranbarri, A., Martínez, M.D., Ayerdi, M., Álvarez, J., Santa-Marina, L., Dorronsoro, M., Ibarluzea, J., 2015. Exposure to fine particle matter, nitrogen dioxide and benzene during pregnancy and cognitive and psychomotor developments in children at 15months of age. Environ. Int. 80, 33–40. https://doi.org/10.1016/j.envint.2015.03.007.
Lertxundi, A., Martinez, M.D., Ayerdi, M., Alvarez, J., Ibarluzea, J.M., 2010. Evaluación de la calidad del aire en medios urbanos de Gipuzkoa. Gac. Sanit. 24, 187–192. https://doi.org/10.1016/j.gaceta.2009.12.005.
Levi, M., Hjelm, C., Harari, F., Vahter, M., 2018. ICP-MS measurement of toxic and essential elements in human breast milk . A comparison of alkali dilution and acid digestion sample preparation methods. Clin. Biochem. 53, 81–87. https://doi.org/10.1016/j.clinbiochem.2017.12.003.
Lin, C.-M.C.-C., Chen, Y.-C., Su, F.-C., Lin, C.-M.C.-C., Liao, H.-F., Hwang, Y.-H., Hsieh, W.-S., Jeng, S.-F., Su, Y.-N., Chen, P.-C., 2013. In utero exposure to environmental lead and manganese and neurodevelopment at 2 years of age. Environ. Res. 123, 52–57. https://doi.org/10.1016/J.ENVRES.2013.03.003.
Lucchini, R.G., Aschner, M., Kim, Y., Šarić, M., 2015. Manganese. In: Nordberg, G., Fowler, B., Nordberg, M. (Eds.), Handbook on the Toxicology of Metals. Elsevier, pp. 975–1011. https://doi.org/10.1016/B978-0-444-59453-2.00045-7.
Mora, A.M., Córdoba, L., Cano, J.C., Hernandez-Bonilla, D., Pardo, L., Schnaas, L., Smith, D.R., Menezes-Filho, J.A., Mergler, D., Lindh, C.H., Eskenazi, B., Van, B., De Joode, W., 2018. Prenatal mancozeb exposure, excess manganese, and neurodevelopment at 1 Year of age in the infants' environmental health (ISA) study. Enviromental Heal. Perspect. 126, 1–9. https://doi.org/10.1289/EHP1955.
Muñoz-Rocha, T.V., Tamayo y Ortiz, M., Romero, M., Pantic, I., Schnaas, L., Bellinger, D., Claus-Henn, B., Wright, R., Wright, R.O., Téllez-Rojo, M.M., 2018. Prenatal co-exposure to manganese and depression and 24-months neurodevelopment. Neurotoxicology 64, 134–141. https://doi.org/10.1016/j.neuro.2017.07.007.
Nandakumaran, M., Al-sannan, B., Al-sarraf, H., Al, M., Nandakumaran, M., Al-sannan, B., Al-sarraf, H., Al-shammari, M., 2016. Maternal – fetal transport kinetics of manganese in perfused human placental lobule in vitro. J. Matern. Neonatal Med. 29, 274–278. https://doi.org/10.3109/14767058.2014.998193.
Ode, A., Rylander, L., Gustafsson, P., Lundh, T., Källén, K., Olofsson, P., Ivarsson, S.A., Rignell-Hydbom, A., 2015. Manganese and selenium concentrations in umbilical cord serum and attention deficit hyperactivity disorder in childhood. Environ. Res. 137, 373–381. https://doi.org/10.1016/j.envres.2015.01.001.
Oulhote, Y., Mergler, D., Barbeau, B., Bellinger, D.C., Bouffard, T., Brodeur, M.-È., Saint-Amour, D., Legrand, M., Sauvé, S., Bouchard, M.F., 2014. Neurobehavioral function in school-age children exposed to manganese in drinking water. Environ. Health Perspect. 122, 1343–1350. https://doi.org/10.1289/ehp.1307918.
Palau Miguel, M., Ligia Guevara Alemany, E., Moreno Seisdedos, M., 2008. Calidad de agua de consumo humano en España. Informe técnico. Trienio 2005-2006-2007. (Madrid).
Perelló, G., Vicente, E., Castell, V., Llobet, J.M., Nadal, M., Domingo, J.L., 2015. Dietary intake of trace elements by the population of Catalonia (Spain): results from a total diet study. Food Addit. Contam. A 32, 1–8. https://doi.org/10.1080/19440049.2015.1018844.
R Core Team, 2017. R: A Language and Environment for Statistical Computing.
Rose, M., Baxter, M., Brereton, N., Baskaran, C., 2010. Dietary exposure to metals and other elements in the 2006 UK total diet study and some trends over the last 30 years. Food Addit. Contam. Part A Chem. Anal. Control. Expo. Risk Assess. 27, 1380–1404. https://doi.org/10.1080/19440049.2010.496794.
Rubio, C., Gutiérrez, Á.J., Revert, C., Reguera, J.I., Burgos, A., Hardisson, A., 2009. Daily dietary intake of iron , copper , zinc and manganese in a Spanish population. Int. J. Food Sci. Nutr. 60, 590–600. https://doi.org/10.3109/09637480802039822.
Santamaria, A.B., Sulsky, S.I., 2010. Current issues risk assessment of an essential Element : manganese. J. Toxicol. Environ. Health 73, 37–41. https://doi.org/10.1080/15287390903337118.
Spencer, A., 1999. Whole blood manganese levels in pregnancy and the neonate. Nutrition 15, 731–734.
Takser, L., Lafond, A.J., Bouchard, M., St-amour, G., Mergler, D., 2004. Manganese levels during pregnancy and at birth : relation to environmental factors and smoking in a Southwest Quebec population. Environ. Res. 95, 119–125. https://doi.org/10.1016/j.envres.2003.11.002.
Takser, L., Mergler, D., Hellier, G., Sahuquillo, J., Huel, G., 2003. Manganese, monoamine metabolite levels at birth, and child psychomotor development. Neurotoxicology 24, 667–674. https://doi.org/10.1016/S0161-813X(03)00058-5.
Vioque, J., Navarrete-Muñoz, E.-M., Gimenez-Monzó, D., García-de-la-Hera, M., Granado, F., Young, I.S., Ramón, R., Ballester, F., Murcia, M., Rebagliato, M., Iñiguez, C., 2013. Reproducibility and validity of a food frequency questionnaire among pregnant women in a Mediterranean area. Nutr. J. 12, 26. https://doi.org/10.1186/1475-2891-12-26.
Wasserman, G.A., Liu, X., Parvez, F., Ahsan, H., Factor-Litvak, P., van Geen, A., Slavkovich, V., Lolacono, N.J., Cheng, Z., Hussain, I., Momotaj, H., Graziano, J.H., 2006. Water arsenic exposure and children's intellectual function in Araihazar, Bangladesh. Environ. Health Perspect. 112, 1329–1333. https://doi.org/10.1289/ehp.6964.
World Health Organization, 1996. Trace Elements in Human Nutrition and Health World Health Organization. World Health Organization, Geneva.
Yoon, M., Schroeter, J.D., Nong, A., Taylor, M.D., Dorman, D.C., Andersen, M.E., Iii, H.J.C., 2011. Physiologically based pharmacokinetic modeling of fetal and neonatal manganese exposure in Humans : describing manganese homeostasis during development. Toxicol. Sci. 122, 297–316. https://doi.org/10.1093/toxsci/kfr141.
Yu, Xiaodan, Cao, L., Yu, Xiaogang, 2013. Elevated cord serum manganese level is associated with a neonatal high ponderal index. Environ. Res. 121, 79–83. https://doi.org/10.1016/j.envres.2012.11.002.
Zheng, W., Fu, S.X., Dydak, U., Cowan, D.M., 2011. Biomarkers of manganese intoxication. Neurotoxicology 32, 1–8. https://doi.org/10.1016/j.neuro.2010.10.002. Biomarkers.
Zota, A.R., Ettinger, A.S., Bouchard, M., Amarasiriwardena, C.J., Schwartz, J., Hu, H., Wright, R.O., 2008. Maternal blood manganese levels and infant birth weight Ami. Epidemiology 20, 1–7. https://doi.org/10.1097/EDE.0b013e31819b93c0.

5.2 ARTÍCULO II

Soler-Blasco, R., Murcia, M., Lozano, M., Sarzo, B., Esplugues, A., Vioque, J., Lertxundi, N., Santa Marina, L., Lertxundi, A., Irizar, A., Braeuer, S., Goesler, W., Ballester, F., Llop, S. (2021). Urinary arsenic species and methylation efficiency during pregnancy: concentrations and associated factors in Spanish pregnant women. Environmental Research, 110889.

Disponible en: https://doi.org/10.1016/j.envres.2021.110889

Resumen

Introducción: El arsénico (As) se considera tóxico para los seres humanos y las principales vías de exposición son el agua potable y la dieta. Una vez ingerido, el arsénico inorgánico se puede metilar secuencialmente a monometil y dimetil arsénico. Varios factores pueden afectar tanto la exposición al As como la eficiencia en la metilación. **Objetivos:** Describir las concentraciones urinarias de las diferentes especies de As y evaluar la eficiencia de metilación durante la gestación, así como sus factores asociados en una cohorte de nacimiento de gestantes españolas. **Métodos**: en este estudio transversal participaron 1.017 mujeres embarazadas de dos zonas de España participantes en el proyecto INMA (Medio Ambiente e Infancia) (2003-2008). Se midió As total (compuestos orgánicos e inorgánicos) y sus principales metabolitos (ácido monometilarsónico, [MMA], ácido dimetilarsínico, [DMA], As inorgánico [iAs]) y arsenobetaína [AB]) en muestras de orina recogidas durante el primer trimestre. La información sociodemográfica y dietética se recopiló mediante cuestionarios. Se utilizaron modelos de regresión lineal multivariante para explorar la asociación entre las concentraciones de especies de As y las covariables. La eficiencia de metilación del As se determinó a través de los porcentajes de los metabolitos y utilizando fenotipos de metilación As, obtenidos mediante el análisis de componentes principales. **Resultados**: Las medianas de las concentraciones fueron 33,0, 21,6, 6,5, 0,35 y 0,33 µg/creatinina para el As total, AB, DMA, MMA e iAs, respectivamente. El consumo diario de arroz y pescado durante el primer trimestre del embarazo se asoció positivamente con la concentración de especies de As (β e intervalo de confianza al 95% [IC95%] = 0,36 [0,09 a 0,64] para arroz e iAs, y 1,06 [0,68 a 1,44] para pescado y AB). Las concentraciones de TA, AB e iAs, y las concentraciones de DMA y MMA se asociaron con el consumo de legumbres y verduras, respectivamente. Las medianas del porcentaje de metabolitos de As fueron 89,7 para %DMA, 5,1 para %MMA y 4,7 para %iAs. Las mujeres no fumadoras y aquellas con mayor índice de masa corporal presentaron una mayor eficiencia de metilación (denotado por un mayor %DMA y menor % MMA). **Conclusión**: Se observó que ciertos factores dietéticos, de estilo de vida y ambientales influyeron tanto en las concentraciones de especies de As como en la eficiencia de metilación en nuestra población. Se necesitan más estudios de cohortes de nacimiento en áreas de baja exposición para mejorar el conocimiento sobre la exposición al arsénico, especialmente a formas inorgánicas, y su posible impacto en la salud durante la infancia.

Environmental Research 196 (2021) 110889

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Urinary arsenic species and methylation efficiency during pregnancy: Concentrations and associated factors in Spanish pregnant women

Raquel Soler-Blasco [a], Mario Murcia [a,b], Manuel Lozano [a,c], Blanca Sarzo [d], Ana Esplugues [a,e,f], Jesús Vioque [g], Nerea Lertxundi [h,i], Loreto Santa Marina [e,h,j], Aitana Lertxundi [e,h,k], Amaia Irizar [h,k,*], Simone Braeuer [l], Walter Goesler [l], Ferran Ballester [a,e,f], Sabrina Llop [a,e]

[a] Epidemiology and Environmental Health Joint Research Unit, FISABIO–Universitat Jaume I–Universitat de València, Valencia, Spain
[b] Health Information Systems Analysis Service, Conselleria de Sanitat, Generalitat Valenciana, Valencia, Spain
[c] Preventive Medicine and Public Health, Food Sciences, Toxicology and Forensic Medicine Department, Universitat de València, Valencia, Spain
[d] Foundation for the Promotion of Health and Biomedical Research in the Valencian Region, FISABIO-Public Health, Valencia, Spain
[e] Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Madrid, Spain
[f] Department of Nursing, Universitat de València, Valencia, Spain
[g] Alicante Institute for Health and Biomedical Research, ISABIAL-UMH, 03010, Alicante, Spain
[h] Biodonostia Health Research Institute, San Sebastian, Spain
[i] Faculty of Psychology of the University of the Basque Country, UPV/ EHU, San Sebastian, Spain
[j] Public Health Division of Gipuzkoa, Basque Government, San Sebastian, Spain
[k] Department of Preventive Medicine and Public Health of the University of the Basque Country, UPV/EHU, Leioa, Spain
[l] Institute of Chemistry, University of Graz, Graz, Austria

ARTICLE INFO

Keywords:
Arsenic
Prenatal exposure
Pregnancy
Cohort studies
Arsenic metabolism

ABSTRACT

Background: Arsenic (As) is considered to be toxic for humans, the main routes of exposure being through drinking water and the diet. Once ingested, inorganic arsenic can be methylated sequentially to monomethyl and dimethyl arsenicals. Several factors can affect both As exposure and methylation efficiency.
Objectives: To describe the urinary concentrations of the different As species and evaluate the methylation efficiency during pregnancy, as well as their associated factors in a birth cohort of pregnant Spanish women.
Methods: Participants in this cross-sectional study were 1017 pregnant women from two areas of Spain who had taken part in the INMA (Environment and Childhood) project (2003–2008). Total As (organic and inorganic compounds) and its main metabolites (monomethylarsonic acid, [MMA], dimethylarsinic acid, [DMA], inorganic As [iAs]) and arsenobetaine [AB]) were measured in urine samples collected during the first trimester. Sociodemographic and dietary information was collected through questionnaires. Multivariate linear regression models were used to explore the association between As species concentrations and covariates. Arsenic methylation efficiency was determined through the percentages of the metabolites and using As methylation phenotypes, obtained from principal component analysis.
Results: Median urine concentrations were 33.0, 21.6, 6.5, 0.35 and 0.33 μg/g creatinine for total As, AB, DMA, MMA and iAs, respectively. Daily consumption of rice and seafood during the first trimester of pregnancy were positively associated with the concentration of As species (i.e., β [CI95%] = 0.36 [0.09, 0.64] for rice and iAs, and 1.06 [0.68, 1.44] for seafood and AB). TAs, AB and iAs concentrations, and DMA and MMA concentrations were associated with legume and vegetable consumption, respectively. The medians of the percentage of As metabolites were 89.7 for %DMA, 5.1 for %MMA and 4.7 for %iAs. Non-smoker women and those with higher body mass index presented a higher methylation efficiency (denoted by a higher %DMA and lower %MMA).

Abbreviations: AB, Arsenobetaine; As, arsenic; AS3MT, Arsenic [+3 oxidation state] methyltransferase gene; BMI, body Mass Index; Cd, cadmium; CI, confident intervals; DMA, dimethylarsinic acid; EFSA, European Food Safety Authority; FFQ, food frequency questionnaire; GM, geometric mean; GSH, glutathione; HPLC, high-performance liquid chromatography; iAs, inorganic arsenic; ICP-MS, inductively coupled plasma mass spectrometry; INMA, INfancia y Medio Ambiente (Environment and Childhood); LOD, limit of detection; MMA, monomethylarsonic acid; Mn, manganese; oAs, organic Arsenic; OCM, one-carbon metabolism; PCA, principal component analysis; SAM, S-adenosylmethionine; SD, standard deviation; Se, selenium; TAs, total arsenic; ΣAs, sum of iAs DMA and MMA; w.w., wet weight; WHO, World Health Organization; Zn, zinc.

* Corresponding author. Biodonostia Health Research Institute, Paseo Doctor Begiristain, s/n, 20014, San Sebastián, Spain.
E-mail address: a.irizarloibide@euskadi.eus (A. Irizar).

https://doi.org/10.1016/j.envres.2021.110889
Received 28 September 2020; Received in revised form 2 February 2021; Accepted 11 February 2021
Available online 17 February 2021

Discussion: Certain dietary, lifestyle, and environmental factors were observed to have an influence on both As species concentrations and methylation efficiency in our population. Further birth cohort studies in low exposure areas are necessary to improve knowledge about arsenic exposure, especially to inorganic forms, and its potential health impact during childhood.

1. Introduction

Arsenic (As) is a toxicant that appears naturally in the soil; however, several human activities also contribute to the presence of As in the environment (European Food Safety Authority, 2009; Fowler et al., 2015). Arsenic can be classified into two groups: organic and inorganic compounds, the inorganic forms being the more toxic species. In some areas of Bangladesh, India, Vietnam, China, Argentina, Chile, Mexico, Australia and USA, the levels of As in drinking water are above the maximum guideline value recommended by the World Health Organization (WHO) in 2003 (10 μg/L) (World Health Organization, 2017), water consumption being the main exposure route for iAs. In regions with low levels of As in water, such as Spain, the main source of exposure to inorganic As (iAs) is rice consumption (European Food Safety Authority, 2014). Regarding organic As (oAs), the main contributor is the consumption of seafood. Arsenobetaine (AB) is usually the major form of arsenic in fish and other seafood. This compound and other oAs forms, such as arsenosugars and arsenolipids, are generally considered less toxic than iAs, although in vitro studies revealed cytotoxic effects of certain arsenic-containing hydrocarbons (Bornhorst et al., 2020).

Arsenic absorbed by the gastrointestinal tract is biotransfomed mainly in the liver (Drobná et al., 2010). Currently, the biotransformation mechanism is not entirely clear, and several possible pathways have been described (Cullen, 2014). The classic pathway proposed by Challenger (1945) was based on the enzymatic reduction and oxidative methylation of As. In this scheme, ingested iAs is reduced (from arsenate -iAs^{V}- to arsenite -iAs^{III}-) and methylated to monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA), S-adenosylmethionine (SAM) being the main donor of the methyl group (Cullen, 2014; Howe et al., 2014). After this biotransformation process, iAs is excreted through the urinary system. In general, the most frequent proportions of the metabolites observed in urine are 60–80% of DMA, 10–20% of MMA and 10–30% of iAs (Vahter, 1999). These relative concentrations reflect the iAs methylation efficiency, indicated by a high %DMA and lower %MMA and %iAs, especially in populations exposed to high levels of iAs through drinking water (Vahter, 1999). This methylation process is considered to be a detoxification mechanism, although high reactivity and toxicity of intermediate compounds, such as MMA, has been demonstrated (Vahter, 2002). Several factors can influence the methylation efficiency, such as age, sex, alcohol and tobacco consumption (Shen et al., 2016; Tseng, 2008). Some nutritional factors seem to increase the efficiency of iAs methylation, especially micronutrients involved in one-carbon metabolism (OCM), such as vitamins B_6 and B_{12}, betaine, choline and folic acid (Bozack et al., 2019; Gamble et al., 2006; Heck et al., 2007; Howe et al., 2017a; Kurzius-Spencer et al., 2017; Laine et al., 2018; Spratlen et al., 2017). Likewise, other experimental and epidemiological studies have shown that some elements, such as selenium (Se), manganese (Mn), zinc (Zn), or cadmium (Cd) can have an influence on As metabolism or interact with this metalloid (Nordberg et al., 2005; Rahman et al., 2019; Sun et al., 2014; Valeri et al., 2016). Regarding organic forms, AB is excreted unchanged in urine, but other organic forms, such as the arsenosugar and arsenolipid species present in seafood, seem to be metabolized and produce DMA (European Food Safety Authority, 2009; Molin et al., 2012; Taylor et al., 2017a).

The effects of As exposure have been studied extensively. In 2009, As and iAs compounds were classified as carcinogenic to humans (International Agency for Research on Cancer, 2012). Other health effects described in adults have been an increased risk of respiratory, cardiovascular, and metabolic diseases (Agency for Toxic Substances and Disease Registry, 2016; Moon et al., 2012; Sanchez et al., 2016). Furthermore, a lower As methylation efficiency has been related to a higher risk of skin lesions, bladder, lung and skin cancer, and peripheral vascular disease (Gamboa-loira et al., 2017; Tseng, 2007). The fetus could be exposed to this toxicant due to transfer of maternal As across the placenta (Gossai et al., 2015). Methylation efficiency has been observed to be augmented during pregnancy, thus increasing the excretion of DMA (Hopenhayn et al., 2003). Nevertheless, several studies have identified adverse effects on fetal and child development caused by prenatal exposure to As, such as miscarriage, low birth weight, skin lesions, respiratory effects, and impairment of neuropsychological development (Freire et al., 2018; Hamadani et al., 2011; Parajuli et al., 2013; Quansah et al., 2015; Sanchez et al., 2016).

Therefore, the aim of this study is to describe the concentrations of total As (TAs) and the different urinary As species (DMA, MMA, AB and iAs) and the methylation efficiency in a birth cohort of pregnant Spanish women. Additionally, we have studied the factors (nutritional, sociodemographic and lifestyle variables) associated with both As exposure and methylation efficiency.

2. Materials and methods

2.1. Study population

In this cross-sectional study, subjects were pregnant women participating in the INMA (Environment and Childhood) Project, a multicentre birth cohort study that aims to investigate the effect of environmental exposures and diet during pregnancy and childhood on fetal and child development in different geographical areas of Spain (http://www.proyectoinma.org).

The study protocol has been reported elsewhere (Guxens et al., 2012). Briefly, 1465 pregnant women were recruited during their first antenatal visit (2003–2008) in two regions of Spain: Gipuzkoa (north of Spain, n=638) and Valencia (east of Spain, n=855). The inclusion criteria were: at least 16 years of age, 10–13 weeks of gestation, singleton pregnancy, intention of undergoing follow-up and delivery in the corresponding centre of reference, and no impediment for communication. These women were monitored during pregnancy. The final study population was made up of 1017 mothers with available urinary arsenic species concentrations at the first trimester of pregnancy (69.4% of total recruited participants). These mothers were selected taking into account two criteria: 1) availability of longitudinal information about their children until 2 years old (in order to assess the potential health effects of prenatal As exposure in further studies), and 2) from the women who met the first criterion we randomly selected a subsample of 1017 due to limited funding for the As analysis.

The study protocol was approved by the Ethics Committee of the university hospital La Fe (Valencia), the Ethics Committee of the Public Health Research Centre in Valencia (CSISP) and the Ethics Committee of Donostia Hospital (Gipuzkoa). Informed consent was obtained from all participants in each phase.

2.2. Study variables and sources of information

2.2.1. Outcome variable: urinary arsenic speciation analysis

Concentrations of total As and its metabolites were determined in spot urine samples collected in the first trimester of pregnancy (mean [SD]= 13.0 [1.2] weeks of gestation). Urine samples were kept frozen at −80 °C until analysis.

The total As concentrations were determined with an inductively coupled plasma tandem mass spectrometer (ICPMS/MS, 8800, Agilent Technologies, Waldbronn, Germany) with oxygen as the reaction gas at m/z 75 → 91. An external calibration was used for quantification, from 0.05 to 100 μg As/L. The certified reference materials (CRM) SRM 1640a (Trace elements in natural water, NIST, Gaithersburg, USA, n=12) and SRM 2669 I and II (Arsenic Species in Frozen Human Urine, n=14) were used for quality control. A calibration standard was re-measured after every 10th sample to monitor the stability of the measurement.

Chromatographic separation of the arsenic compounds was carried out in accordance with a previously validated method (Scheer et al., 2012). An external calibration was used for quantification. It contained arsenate, MMA, DMA and AB in the concentration range 0.05–100 μg As/L. Hydrogen peroxide (10% v/v) was added to oxidize the species. For quality control, the CRMs SRM 1640a (n= 5), SRM 2669 I (n = 8) and SRM 2669 II (n = 22) were prepared similar to the urine samples and also investigated. The 1.0 μg As/L calibration standard was injected regularly to control the stability of the measurement. Every 10th sample was also re-measured for the same purpose. Of all the samples analysed (1017), 102 were re-analysed, which represents 10%. These second measurements matched the first in 100 ± 3% of the cases.

HPLC (1200, Agilent Technologies) coupled to ICPMS/MS (8800, Agilent Technologies) was employed for speciation analysis. The arsenic signal was again recorded in oxygen reaction mode at m/z 75 → 91, with the addition of CO2 for signal enhancement.

TAs concentrations and the sum of all As species were compared. When the difference was larger than 15%, either the total arsenic or the speciation analysis was repeated (124 samples).

Limits of detection (LOD) of AB, DMA, MMA and iAs for the samples from Valencia were 0.02, 0.02, 0.03 and 0.03 μg/L and for the Gipuzkoa samples they were 0.02, 0.03, 0.03 and 0.02 μg/L. When samples were below LOD, ½ LOD was assigned for the statistical analysis (2.8% of samples for MMA levels and 2.3% of samples for iAs levels).

2.2.2. Covariates

2.2.2.1. Sociodemographic variables. Women filled in two questionnaires during their pregnancy, at the first and the third trimesters of gestation (mean [SD]= 13.1 [1.5] and 32.5 [2.2] weeks of gestation, respectively). The questionnaires were administered by trained interviewers and focused on sociodemographic, environmental and lifestyle information. In both cohorts, the questionnaires were offered in Spanish and, additionally, in the co-official language (Basque) in the Gipuzkoa cohort. We selected the covariates used in this study from the previous literature on this topic (European Food Safety Authority, 2014; Nigra et al., 2019; Saxena et al., 2018; Shen et al., 2016; Tseng, 2008): age at conception (years), education level (up to primary, secondary, university), place of birth (Spain, Latin America, other), body mass index (BMI, kg/m^2) before pregnancy (continuous and categorized by low and healthy weight [<25], overweight [25 ≤ 30], obesity [≥30]), parity (0, ≥1), working status at first trimester of pregnancy (non-worker, worker), area of residence (rural, non-rural), tobacco and alcohol consumption in the first trimester of pregnancy (yes, no), and season of sample collection (spring, summer, winter, autumn). Subjective variables about the proximity of the residence to industrial or agricultural areas (yes, no) and frequency of traffic near the residence (continuous, quite frequent, infrequent/never) were collected.

We defined parental social class from the maternal or paternal occupation during pregnancy with the highest social class, according to the Spanish adaptation of the International Standard Classification of Occupations coding system approved in 1988 (ISCO88). Class I + II included managerial jobs, senior technical staff, and commercial managers; Class III included skilled non-manual workers; and class IV + V included manual and unskilled workers.

2.2.2.2. Dietary variables. Information on diet during the first trimester of pregnancy was obtained from a 100-item semi-quantitative food frequency questionnaire (FFQ) completed at the time of sampling. The dietary information covered the time from the last menstruation to the first prenatal visit, which occurred between weeks 10 and 13 of pregnancy. This FFQ was validated with good reproducibility for nutrient and food intake (Vioque et al., 2013). The items had nine possible responses, ranging from 'never or less than once per month' to 'six or more per day'. A commonly used serving size was specified for each food item in the FFQ. This was converted to average daily intake in grams for each individual participant. We obtained data on the intake of dairy products, eggs, meat, seafood and shellfish, fruits, vegetables, legumes, nuts, potatoes, cereals and bread, and coffee and other infusions. Some foods were explored in a more comprehensive manner due to their clear relationship with As concentrations, as is the case of rice, other cereals and bread, fish split into categories (lean fish, oily fish and shellfish and molluscs), and meat split into categories (red and white meat) (European Food Safety Authority, 2009). Moreover, we obtained information about consumption of tap water (less than 1 glass [250 cc] a day, 1 or more glasses a day).

Additionally, dietary folate, folic acid, vitamins B_{12} and B_6, iron (Fe) and Zn intake were estimated using the food composition tables of the US Department of Agriculture (U.S. Department of Agriculture: Agricultural Research Service USDA, 2007) and with Spanish sources (Palma et al., 2008). Energy-adjusted intakes were computed using the residual method (Willet, 2013). Information on the intake of supplements (brand name, dose and composition) was also collected, converted into nutrient intake daily dose, and added to the calculation of total daily nutrient intake (Vioque et al., 2013). Moreover, categorical variables were created for estimated vitamins B_{12} and B_6, folate, Fe and Zn intake (<or ≥ the Population Reference Intake for Fe, Zn and vitamin B_6 and adequate intake for folate and vitamin B_{12}) (European Food Safety Authority, 2019).

2.2.2.3. Other elements. Mn and Se concentrations were determined in serum samples taken at the first trimester of pregnancy. More information about the methodology has been reported in detail elsewhere (Lozano et al., 2020; Soler-Blasco et al., 2020). Cd concentrations were determined by ICPMS in urine samples taken at the first trimester of pregnancy. Creatinine concentrations were measured in the same urine samples at the first trimester of pregnancy by DRI® Creatinine-Detected® Test using AV680 from Beckman Coulter. The quantification of maternal plasmatic levels of ferritin was performed by fluoroimmunoassay (DELFIA Ferritin kit A069-101), in the Gipuzkoa Public Health Laboratory. In Valencia, the quantification was performed at La Fe hospital through immunoturbidimetry in Beckman Coulter AU analysers (Arija et al., 2019).

2.3. Statistical analysis

Descriptive and bivariate analyses were performed using Fisher's Exact Test for categorical variables and the Kruskall Wallis Test for continuous variables in order to detect any differences between the included and the excluded populations.

In populations with moderate-high fish/seafood consumption, the ΣAs could not properly reflect the exposure to inorganic As, because oAs from seafood contributes to DMA levels and TAs (Navas-Acien et al., 2011). To eliminate the influence of seafood arsenicals, we calibrated the methylated and non-methylated species concentrations using a mathematical method proposed by Jones et al. (2016). In brief, AB concentrations were used as a marker of seafood consumption. Using linear regression models, calibrated iAs, DMA and MMA concentrations were estimated by regressing the measured concentrations of iAs, MMA, and DMA on AB and creatinine concentrations (all measures were log-2 transformed) in three separate models. The new calibrated iAs, MMA

and DMA concentrations were calculated by adding the residual of each metabolite model to a constant (mean level of each metabolite estimated from participants with AB<1 μg/L).

We calculated the geometric mean (GM) and 95% confidence intervals (95%CI) of the urinary TAs, AB, DMA, MMA, iAs, and ΣAs (as the sum of iAs, DMA and MMA]) for both measured and calibrated concentrations. Additionally, GM and 95%CI of the measured concentrations were calculated according to sociodemographic, environmental and dietary characteristics of the study population. Concentrations were expressed in μg/L and corrected to creatinine content (μg/creatinine). The GMs were compared using the ANOVA F-test. For further analysis, we used the log2 and probit-transformed values of the urinary As species concentrations and the percentage of the individual metabolites, respectively, to approach normality.

Bivariate and multivariate linear regression models were built in order to study the relationship between urinary As concentrations and the sociodemographic, environmental, and dietary factors. Beta coefficients (β) and 95%CI were obtained. A two-step procedure was used to construct the multivariate models. First, core models were built using all the sociodemographic and environmental covariates associated with a p value < 0.2 in the bivariate analysis. Following a backward elimination procedure, the covariates associated with each species at a level of p value < 0.1 in the likelihood ratio test were retained in the model. Second, each food group was adjusted in the core model individually. The final multivariate models were built using the core model and all the food groups associated with each species at a level of p value < 0.1 in the likelihood ratio test. Although food intake or estimated nutrients variables were mutually correlated (Fig. S1 and Fig. S2), we found no collinearity among them in the final models. The area of study (Valencia or Gipuzkoa) and urinary creatinine levels in the first trimester of pregnancy were included in all the models regardless of their statistical significance.

Methylation As efficiency was determined through two approaches. First, by calculating the percentage of the individual calibrated metabolites (iAs, MMA and DMA) over the sum of those species (ΣAs). Second, a principal component analysis (PCA) of the three calibrated, untransformed and un-rotated percentages was performed. The main reason for carrying out a PCA was to avoid the high correlation between the percentages of the three metabolites, by transforming the three interrelated variables into two independent measures of As metabolism phenotypes (Balakrishnan et al., 2016; Gribble et al., 2015; Jansen et al., 2016; Spratlen et al., 2017). The results obtained through the PCA analysis were two principal components (PC1 and PC2) that explained 100% of the original variance. Therefore, these PC were used as As methylation phenotypes.

We analysed the factors associated with the methylation efficiency using the same procedure as described above. In particular, we performed five different models by using the probit-transformed calibrated %DMA, %MMA, %iAs and untransformed PC1 and PC2 as outcome variables. In these models, the variables used in the second step were the estimation of nutrients and vitamin intake described above and Se, Mn and Cd concentrations. The area of study was also included.

To check whether linear regression assumptions were met, we visually inspected model residuals for normality and homoscedasticity. No influential data were identified by Cook's distance. Variance inflation factors (VIFs) were used to test for collinearity among variables in the final models, all VIFs being <2.5. To deal with the possibility of minor deviations from normality and homoscedasticity, confidence intervals were calculated on the basis of robust standard error in the final results.

Statistical analyses were carried out using R statistical package version 3.5.1 (R Core Team, 2017).

3. Results

3.1. TAs and As metabolite concentrations and associated factors

Differences between included and excluded subjects are shown in Table S1. Among the participants, there was a higher percentage of slightly older women with a higher level of education and higher social class than among the excluded women. There were no differences in rice consumption. Participants showed higher fish consumption than the excluded women. Among the participants, there was a slightly lower percentage of women who did not take any vitamin supplement during the period of the study (4% vs. 7%).

The GM [95%CI] of measured urinary TAs, ΣAs and AB concentrations were 35.55 [33.10–38.19], 7.74 [7.41–8.09] and 20.17 [18.34–22.19] μg/g creatinine, respectively. Table 1 shows measured and calibrated As concentrations and the metabolite percentages. The calibrated concentrations of metabolites were lower in all cases, especially for DMA concentrations (GM [95%CI] 6.82 [6.52, 7.14] μg/g creatinine and 2.98 [2.85, 3.09] μg/g creatinine, in measured and calibrated DMA concentrations, respectively). Correlations between measured and calibrated metabolite concentrations were high (Pearson correlation coefficient of 0.85 for DMA, and 0.99 for MMA and iAs, p value < 0.01 in all cases). The correlation between DMA and TAs decreased from 0.69 to 0.23 when the metabolite was calibrated **(Fig. S3).**

Table 1
Geometric mean and 95% confidence intervals of measured and calibrated[a] urinary As concentrations and percentages. INMA Project (Valencia and Gipuzkoa, Spain. 2003–2008).

	Measured concentrations		Calibrated concentrations[a]	
As concentrations	μg/L	μg/g creatinine	μg/L	μg/g creatinine
TAs	28.89 (26.81, 31.13)	35.55 (33.10, 38.19)		
AB	16.40 (14.88, 18.08)	20.17 (18.34, 22.19)		
ΣAs	6.28 (5.98, 6.59)	7.74 (7.41, 8.09)	2.94 (2.82, 3.06)	3.62 (3.48, 3.76)
DMA	5.54 (5.27, 5.82)	6.82 (6.52, 7.14)	2.41 (2.31, 2.51)	2.97 (2.85, 3.09)
MMA	0.28 (0.26, 0.29)	0.34 (0.32, 0.36)	0.19 (0.18, 0.20)	0.23 (0.22, 0.24)
iAs	0.27 (0.25, 0.29)	0.33 (0.31, 0.35)	0.21 (0.20, 0.23)	0.26 (0.25, 0.28)
Metabolite percentages				
%DMA[b]	89.7 (89.3, 90.2)		84.4 (83.9, 84.9)	
%MMA[b]	5.1 (4.8, 5.3)		7.1 (6.7, 7.3)	
%iAs[b]	4.7 (4.5,5.0)		7.6 (7.3, 8.0)	

Note: TAs, Total As; ΣAs, sum of DMA, MMA and iAs; DMA, dimethylarsinic acid; MMA, monomethylarsonic acid; iAs, inorganic As; %DMA, percentage of dimethylarsinic acid; %MMA, percentage of monomethylarsonic acid; %iAs, percentage of inorganic As.
The percentages of each metabolite were calculated: levels of the metabolite/(DMA + MMA + iAs)*100; μg/L: micrograms per litre. μg/g creat: micrograms per gram of creatinine.

[a] As metabolite concentrations corrected by arsenobetaine concentrations;
[b] Median (95% confidence intervals).

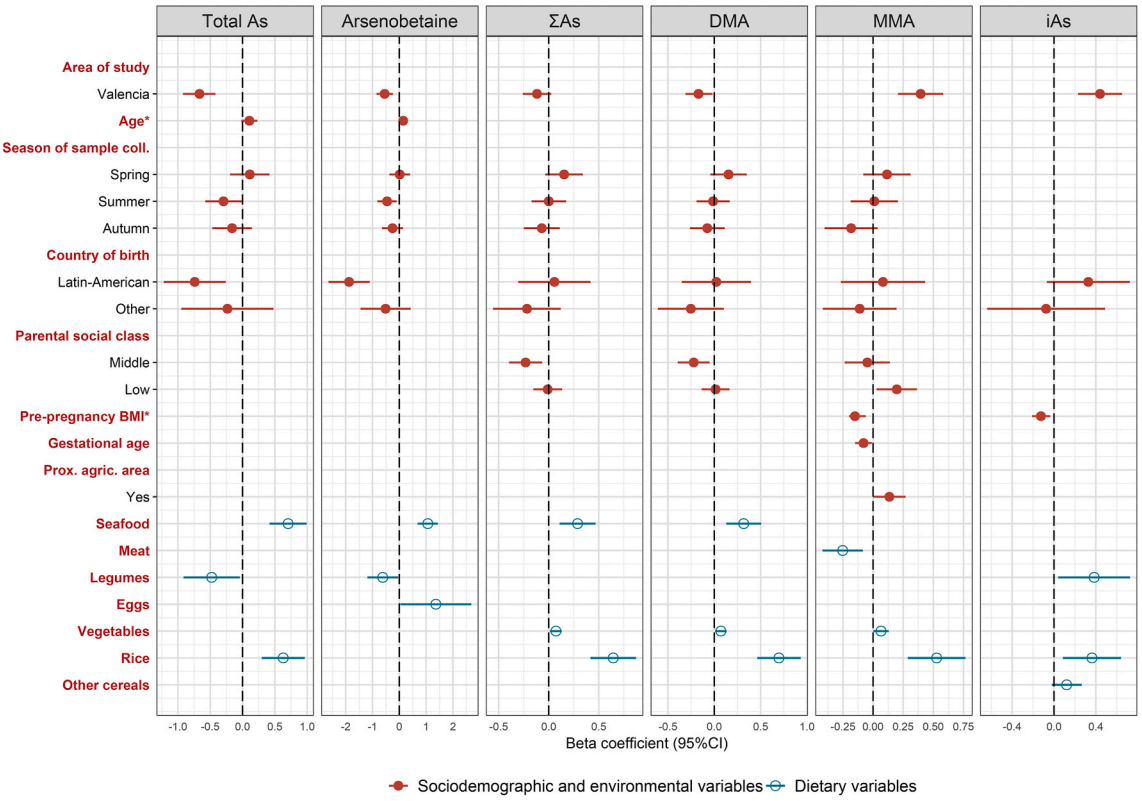


Fig. 1. Beta coefficients (CI95%) of the multivariate linear regression between measured levels of arsenic metabolites in maternal urine, and sociodemographic and dietary factors. INMA Project (Valencia and Gipuzkoa, Spain, 2003–2008). Note: TAs, Total As; ∑As, sum of DMA, MMA and iAs; DMA, dimethylarsinic acid; MMA, monomethylarsonic acid; iAs, inorganic As; ref, category reference; Coll, collection: Prox.agric.area, proximity to agricultural area. Dietary factors are expressed in 100 g per day at first trimester of pregnancy, adjusted for calories. Reference categories: area of study (Gipuzkoa); season of sample collection (winter); maternal country of birth (Spain); parental social class (high); proximity to agricultural area (no). Samples used in all models were n=1005, except for log2 MMA model (n=994). TAs, AB, ∑As, DMA, MMA and iAs concentrations were log2-transformed.*Age and pre-pregnancy BMI variables were expressed as increments of 5 years and 5 kg/m^2, respectively.

Table S2 shows the total As and metabolite concentrations by participant characteristics. Women from Gipuzkoa presented higher TAs and AB concentrations and lower levels of MMA and iAs than those from Valencia. Latin-American women presented higher concentrations of iAs and MMA than the Spaniards, and very low AB levels. The urine samples collected during spring presented higher levels of TAs, ΣAs, DMA, MMA and iAs. Consumption of more than 1 serving of rice per week was associated with an increase in DMA, MMA and iAs concentrations. Consumption of fish (<1 serving per week, 1 serving per week, > 1 serving per week) showed a clearly increasing trend with AB and TAs concentrations.

The multivariate models for the factors associated with the concentrations for each of the measured As species can be observed in Fig. 1. The area of study was significantly related to all As measures, except for ΣAs concentrations. Women from Valencia presented higher concentrations of MMA (β [95%CI]: 0.51 [0.35, 0.68], p value < 0.01) and iAs (β [95%CI]: 0.44 [0.23, 0.65], p value < 0.01), but lower concentrations of DMA (β [95%CI]: 0.17 [-0.31, −0.02], p= 0.03) and AB (β [95%CI]: 0.55 [-0.85, −0.24, p value < 0.01). Latin-American mothers showed lower concentrations of AB and the TAs (β [95%CI]: 1.88 [-2.65, −1.10], p value < 0.01, and −0.74 [-1.22, −0.26], p value =0.01, respectively). Parental social class was related to ΣAs and DMA concentrations, lower levels being observed in middle social class mothers (β [95%CI]: 0.23 [-0.40, −0.07], p value =0.01, and −0.22 [-0.39, −0.05], p value =0.01, respectively). Pre-pregnancy BMI was negatively associated with MMA and iAs concentrations (β [95%CI]: 0.03 [-0.04, −0.01], p value < 0.01, and −0.03 [-0.04, −0.01], p =0.01, respectively). Proximity of residence to an agricultural area was positively and significantly related to MMA concentrations (β [95%CI]: 0.14 [0.001, 0.27], p value =0.05).

Regarding dietary variables, a positive and significant association between seafood consumption and all As measures was observed, except for MMA and iAs concentrations, with a stronger association with AB concentrations (β [95%CI]: 1.06 [0.69, 1.43], p value < 0.01).

Similarly, rice consumption during pregnancy was positively associated with all As measures (β [95%CI]: 0.63 [0.27, 0.99], p value < 0.01 for TAs; 0.64 [0.46, 0.86], p value < 0.01 for ΣAs; 0.70 [0.47, 0.92], p value < 0.01 for DMA; 0.50 [0.26, 0.73], p value < 0.01 for MMA; and 0.36 [0.09, 0.64], p value =0.01 for iAs), except for AB concentrations. Consumption of other cereals was positively associated with iAs concentrations (β [95%CI]: 0.12 [-0.01, 0.25], p value =0.07). The association between the intake of legumes and TAs and AB concentrations was negative (β [95%CI]: 0.48 [-0.90, −0.05], p value =0.03; and −0.62 [-1.17, −0.07], p value =0.03, respectively), but positive for iAs (β [95%

Table 2
Summary of principal components analysis of calibrated[a] percentage As metabolites.

	PC1	PC2
Standard deviation	0.13	0.05
Proportion of variance	0.88	0.12
Weight for calibrated[a] %DMA	0.77	0.27
Weight for calibrated[a] %MMA	−0.15	−0.80
Weight for calibrated[a] %iAs	−0.62	0.53

Note: PC1, principal component 1; PC2, principal component 2; %DMA, percentage of dimethylarsinic acid; %MMA, percentage of monomethylarsonic acid; %iAs, percentage of inorganic As.

[a] As metabolite concentrations corrected for arsenobetaine and creatinine concentrations.

Table 3
Beta coefficient (95%CI) of the multivariate linear regression between methylation efficiency (measured by calibrated[1] percentage of As metabolites in maternal urine and principal component 1 and 2 of PCA) and sociodemographic, estimated nutrients intake (adjusted for calories) and essential and toxics elements factors. INMA Project (Valencia and Gipuzkoa, Spain, 2003–2008).

	Calibrated %DMA[a,b] (n= 1005)		Calibrated %MMA[a,b] (n= 998)		Calibrated %iAs[a,b] (n= 999)		PC1 (n= 992)		PC2 (n= 992)	
	Beta (95%CI)	P[c]	Beta (95%CI)	P[c]	Beta (95%CI)	P[c]	Beta (95%CI)	P[c]	Beta (95%CI)	P[c]
Sociodemographic, environmental and lifestyle variables										
Area of study (ref. Gipuzkoa)										
Valencia	−8.90 (−13.95, −3.85)	<0.01	12.41 (7.22, 17.60)	<0.01	6.55 (0.73, 12.37)	0.02	−0.02 (−0.04, 0.00)	0.03	−0.02 (−0.02, −0.01)	<0.01
Place of birth (ref. Spain)										
Latin America	14.24 (1.50, 26.97)	0.03	−18.28 (−26.98, −9.59)	<0.01					0.03 (0.01, 0.04)	<0.01
Other	−3.87 (−18.13, 11.19)		0.55 (−9.78, 10.88)						0.01 (−0.01, 0.02)	
Working status (ref. non-worker)										
Worker			−4.84 (−9.34, −0.34)	0.05					0.01 (0.01, 0.02)	0.03
Parental social class (ref. I + II high)										
III							0.002 (−0.020, 0.025)	0.08		
IV + V (low)							0.020 (−0.001, 0.040)			
Tobacco consumption (ref. no)										
Yes					6.07 (−0.07, 12.20)	0.08	−0.022 (−0.042, −0.002)	0.03		
Body mass index	1.16 (0.60, 1.72)	<0.01	−0.83 (−1.31, −0.35)	<0.01	−0.94 (−1.52, −0.36)	<0.01	0.003 (0.002, 0.005)	<0.01	0.001 (0.000, 0.001)	0.02
Gestational age at sampling			−1.95 (−4.24, 0.35)	0.05						
Estimated daily intake of nutrients										
Estimated maternal zinc intake (mg/day)[d,e]	7.71 (−1.31, 16.81)	0.09								
Estimated maternal iron intake (mg/day)[d,e]							−0.016 (−0.036, 0.004)	0.02		
Estimated maternal folate ntake (μg/day)[d]									0.002 (0.000, 0.004)	0.01
Other toxic elements										
Maternal urine cadmium (μg/L)[e]			1.89 (0.29, 3.49)	0.02					−0.002 (−0.005, −0.00)	0.03

Note: 95%CI, 95% confidence intervals; %DMA, percentage of dimethylarsinic acid; %MMA, percentage of monomethylarsonic acid; %iAs, percentage of inorganic As. PCA, principal component analysis; PC1, principal component 1; PC2, principal component 2.
The percentages of each metabolite were calculated: levels of calibrated metabolite/(calibrated DMA + calibrated MMA + calibrated unmethylated iAs.
[a] Calibrated percentages were calculated with As metabolite concentrations corrected by arsenobetaine and creatinine concentrations;
[b] Probit-transformed appears
[c] p value from ANOVA F- test.
[d] Estimated daily intake of nutrients from the diet and supplementation.
[e] log2-transformed.Each model was simultaneously adjusted by all the presented variables.

Table 4
Geometric mean urinary arsenic concentrations and their metabolites measured in pregnant women in other published studies.

Study	Location	Gestational Age	N	Year of sampling	ΣAs	iAs	MMA	DMA	AB	%iAs	%MMA	%DMA
Present study	Spain (GIP-VAL)	1st trimester (13 weeks)	1017	2003–2008	7.7 (7.4, 8.1)[c]	0.33 (0.31, 0.359)[c]	0.34 (0.32, 0.36)[c]	6.8 (6.5, 7.1)[c]	20.17 (18.34, 22.19)[c]	4.3 (4.0, 4.5)[c]	4.4 (4.2, 4.6)[c]	88.2 (87.6, 88.7)[c]
Farzan (2020)	US	≤20 wk	241	2015–2019	ND	1.0 (0.6, 1.3)[b,f]	0.5 (0.2, 0.7)[b,f]	4.1 (3.0, 5.6)[b,f]	0.5 (0.2, 1.9)[b,f]	15.8 (10.0, 23.3)[b,f]	8.0 (6.0, 10.3)[b,f]	74.8 (65.9, 81.6)[b,f]
Gao (2019)	Bangladesh	4–16 weeks	1425	2008–2011	ND	6.5 (0, 98.9)[c,i]	3.8 (0, 57.9)[c,i]	65.1 (17.5, 530)[c,i]	ND	8.5 (0, 24)[c,i]	4.9 (0, 13.2)[c,i]	85.7 (66.6, 100)[c,i]
Howe (2020)	US	Early pregnancy (6–24 weeks)	167	2015	5.66 (1.96, 28.75)[a,g]	0.93 (0.17, 9.82)[a,g]	0.44 (0.12, 4.96)[a,g]	4.24 (0.82–21.11)[a,g]	0.50 (0.04, 478.82)[a,g]	15.3 (2.6, 63.4)[a,g]	8.0 (22.5, 94.1)[a,g]	75.1 (22.5, 94.1)[a,g]
Stajnko (2019)	Croatia-Slovenia	3rd trimester	136	2006–2011	3.23 (2.84, 3.68)[b]	As^{III}: 0.11 (0.10, 0.13)[b] As^{V}:<LOD	0.15 (0.13, 0.35)[b]	2.43 (2.06, 2.85)[b]	19.8 (14.8, 26.5)[b]	As^{III} 3.83 (3.16, 4.62)[b]	5.13 (4.40, 5.98)[b]	82.7 (77.2, 88.5)[b]
Ettinger (2017)	Canada	1st trimester	1933	2008–2011	ND	<50% below the LOD	<50% below the LOD	2.57 (2.49, 2.65)[b]	ND	ND	ND	ND
Laine (2015)	Mexico	At delivery	200	2011–2012	23.3 (4.3, 319.7)[b,g]	1.3 (0.14, 23.0[b,g]	1.4 (0.12, 18.2)[b,g]	20.6 (1.4, 292.5)[b,g]	ND	5.3 (0.77, 45.1)[b,g]	6.0 (0.68, 24.9)[b,g]	88.5 (32.7, 96.7)[b,g]
Neamtiu (2015)	Romania		10[j]	2011–2013	5.0 (3.7, 8.9)[b,h]	0.4 (0.4, 1.1)[b,h]	0.5 (0.2, 1.1)[b,h]	4.2 (2.0, 7.2)[b,h]	ND	ND	14.5 (5.9, 28.3)[b,h]	79.1 (52.7, 88.3)[b,h]
			10[k]		6.6 (3.9, 10)[b,h]	0.4 (0.3, 1.1)[b,h]	0.5 (0.4, 1.7)[b,h]	5.5 (3.1, 8.8)[b,h]	ND	ND	12.0 (9.1, 18.5)[b,h]	80.9 (77.2, 87.6)[b,h]
Chou (2014)	China	3rd trimester	299	2001–2002	22.6 (8.21, 36.72)[c,f]	0.79 (0.38, 1.49)[c,f]	0.46 (0.18, 2.03)[c,f]	20.01 (7.48, 32.30)[c,f]	ND	ND	ND	ND
Gilbert-Diamond (2011)	USA	24–28 weeks	229	2009	3.78 (1.80, 6.10)[a,f]	0.24 (0.13, 0.40)[a,f]	0.30 (0.14, 0.50)[a,f]	3.25 (1.51, 5.53)[a,f]	0.67 (0.07–5.47)[a,f]	ND	ND	ND
Gardner (2011)	Bangladesh	8 weeks	324	2001–2003	94 (3.0)[b,d]	ND	ND	ND	ND	16 ± 10[e]	11 ± 4.2[e]	73 ± 11[e]
		14 weeks			112.0 (3.0)[b,d]	ND	ND	ND	ND	14 ± 6.4[e]	7.6 ± 3.1[e]	79 ± 7.5[e]
		30 weeks			103 (2.9)[b,d]	ND	ND	ND	ND	10 ± 4.9[e]	6.5 ± 2.8[e]	83 ± 6.0[e]
Jay Christian (2006).	Chile	During pregnancy (not specified)	93	1998–2000	55.8 (41.6)[a,g]	5.6 (5.5)[a,g]	3.2 (4.0)[a,g]	46.9 (36.1)[a,g]	ND	11.0 (9.7)[a,g]	5.2 (4.7)[a,g]	83.8 (11.0)[a,g]

Note: ND, No data available; LOD, limit of detection; TiAs, Total inorganic iAs (sum of DMA, MMA and iAs); DMA, dimethylarsinic acid; MMA, monomethylarsonic acid; iAs, unmethylated inorganic As.

[a] μg/L (unadjusted).
[b] adjusted by specific gravity.
[c] adjusted by creatinine (μg/g creatinine).
[d] geometric standard deviation.
[e] AM ± standard deviation.
[f] Median (percentile 25, percentile 75).
[g] Median (range).
[h] Median (CI95%).
[i] Median (percentile 5, percentile 95).
[j] Pregnant women exposed to average iAs water concentration <0.5 μg/l.
[k] Pregnant women exposed to average iAs water concentration 10.24 μg/l

CI]: 0.38 [0.06, 0.71]). Meat consumption was negatively associated with MMA concentrations (β [95%CI]: 0.26 [-0.43, −0.09], p value =0.02). When seafood consumption was replaced by the different fish categories (lean fish, oily fish, and shellfish and mollusc consumption), a positive and significant association among the consumption of shellfish and molluscs and TAs, AB, ΣAs, DMA and iAs concentrations was observed (see Fig. S4). Similarly, the meat intake variable was split into two different categories (red and white meat), and only red meat was associated with the MMA metabolite (β [95%CI]: 0.39 [-0.61, −0.18], p value < 0.01) (see Fig. S4).

3.2. Arsenic methylation efficiency and factors associated

The medians (95%CI) of the percentage of As metabolites were 89.7 (89.3, 90.2) for %DMA, 5.1 (4.8, 5.3) for %MMA, and 4.7 (4.5, 5.0) for %iAs. When the percentage of As metabolites was calculated with the calibrated concentration, we observed a decrease in %DMA (84.4 [83.9, 84.9]) and an increase in %MMA and %iAs (7.1 [6.7, 7.3] and 7.6 [7.3, 8.0], respectively) (see Table 1). The variability of these three variables can be summarized by two principal components (Table 2). Principal component 1 (PC1) explained 88% of the variance and reflected higher %DMA, and lower %MMA and %iAs. Principal component 2 (PC2) explained the remaining 12% of the variance and reflected higher %iAs and lower %MMA.

The variables associated with the percentage of each calibrated metabolite in the multivariate model can be observed in Table 3. The area of Valencia was associated with a lower methylation (higher % MMA and %iAs and lower %DMA). Latin-American mothers presented higher %DMA and lower %MMA (β [95%CI]: 14.2 [1.5, 26.3 and −18.5 [-27.3, −9.8], respectively]), but this factor did not remain in the %iAs model. Mothers who worked during pregnancy showed lower %MMA. Tobacco consumption during the first trimester of pregnancy was associated with higher %iAs (β [95%CI]: 6.1 [-0.07, 12.2]). Pre-pregnancy BMI was associated with a higher methylation (lower %MMA and % iAs and higher %DMA). Furthermore, gestational age at sampling was inversely related to %MMA (β [95%CI]: 0.84 [-1.32, −0.36]). Regarding the estimated nutrients, only the estimation of daily intake of Zn was positively associated with %DMA in the bivariate analysis (Table S3), and this association continued to be present in the multivariate model, although in a marginal way (β [95%CI]: 7.71 [-1.39, 16.81]). The micronutrients involved in the one-carbon metabolism evaluated (estimated folate and vitamins B_6 and B_{12} intake) and other elements evaluated (serum Se and Mn concentrations) were not related to As metabolite percentages in the bivariate or the multivariate analyses **(Table S3)**. Higher levels of urinary Cd were associated with higher % MMA (β [95%CI]: 1.89 [0.28, 3.49]).

Finally, the variables associated with the As PC1 and PC2 methylation phenotypes in the multivariate model can be observed in Table 3. Regarding PC1, the area of Valencia, tobacco consumption during pregnancy and a higher estimated Fe intake were negatively associated with PC1. Pre-pregnancy BMI was directly associated with PC1, reflecting a better capacity to produce DMA. The factors associated with PC2 were the area of study (lower among the Valencian women), the place of birth (higher in Latin-American women), working situation during pregnancy (higher among workers) and higher BMI.

4. Discussion

In the present study, we measured the urinary concentrations of As metabolites in a large sample of pregnant women from Spain, a country without high levels of environmental iAs exposure, and high rice and seafood consumption. The main factor contributing to the As concentrations was diet, rice being the main source of exposure to inorganic species (iAs, MMA and DMA) while seafood contributed mainly to DMA and the organic form (AB). We also estimated the methylation efficiency by two different approaches (the relative percentages of each As metabolite and a principal component analysis). The factors that were most clearly associated were area of study, women's place of origin, body mass index and tobacco consumption. When the percentages of calibrated iAs, MMA and DMA were combined into principal components, 88% of the variance was explained by the first of them.

4.1. Concentrations of As and its metabolites during pregnancy: comparison with previous studies

Concentrations of urinary inorganic As species in our study population were lower than those observed in other areas. For example, in Bangladesh or Chile the mean ΣAs concentrations [SD] were 112.0 [3.0] and 55.8 [41.6] μg/L (Gardner et al., 2011; Jay Christian et al., 2006), compared with the GM of 7.7 μg/g creatinine observed in the present study (Table 4). Other populations with higher levels of ΣAs are those from Mexico and China, with median concentrations of 23.3 μg/L and 22.6 μg/g creatinine, respectively (Chou et al., 2014; Laine et al., 2015). These areas present high levels of As in groundwater, due to geochemical natural processes, and also anthropogenic processes, such as mining or smelting (Litter et al., 2020). In our study, urinary concentrations of iAs and MMA were higher than in other studies carried out on regions with low levels of iAs in drinking water, such as some areas of USA, Croatia, Slovenia and Canada (Howe et al., 2020; Stajnko et al., 2019; Vaughan Watson et al., 2020). Conversely, in our study urinary AB concentrations were higher than in other studies conducted in USA, the main reason probably being the high fish and seafood consumption of the Spanish population.

4.2. Factors associated with As exposure: dietary factors

In our study, rice consumption was the main predictor of TAs and all As species, except for AB. A recent study conducted in Valencia (Spain) showed that rice was a foodstuff with high total As and iAs content (0.15 and 0.06 mg/kg, respectively) (Marín et al., 2018). In that study, all the samples were below the limits established by the European regulation (0.20–0.25 mg/kg for iAs) (European Commission, 2015). In another study carried out on the Iberian Peninsula (Spain and Portugal), the As species concentrations were analysed according to the type of rice and geographical region (Signes-Pastor et al., 2016). The results showed that 26% of the rice samples exceeded the levels of iAs established for rice-based food for infants and young children (0.10 mg/kg). The same study found that commercial brown rice was the type that presented the highest levels of iAs and DMA species (0.16 and 0.084 mg/kg, respectively), compared with polished rice, both produced in the Iberian Peninsula region. Spain is the European country with the second highest consumption of rice, after Portugal (11 kg per capita per year) (Food and Agriculture Organization of the United Nations, 2017).

In our population, the consumption of 100 g of seafood increased the urinary concentrations of AB by 109%. It is well-established that AB is the major As species present in fish (European Food Safety Authority, 2009). This organoarsenical is considered non-toxic, because it is rapidly excreted without undergoing any change (European Food Safety Authority, 2009). Additionally, we observed that fish consumption during pregnancy was positively associated with urinary DMA levels. The explanation for this finding could be related to some more complex forms of As, such as arsenosugars and arsenolipids, detected in fish, marine algae and filter feeders (European Food Safety Authority, 2009; Taylor et al., 2017b). It has been suggested that these As forms are biotransformed in the human body into species like DMA (Molin et al., 2012). In relation to the inorganic forms, iAs levels in fish and shellfish are thought to be low (European Food Safety Authority, 2014). Nevertheless, Spain has recently been reported as one of the European countries with the highest iAs intake through fish and seafood, especially among Mediterranean high consumers of molluscs (Ferrante et al., 2019). Indeed, in our population, the consumption of 100 g of shellfish and molluscs was associated with an increase of 146% in urinary iAs

concentrations (CI95%: 38,341%). This increase was stronger than the association with rice consumption (26%, CI95%: 4,52%).

Other food groups associated with the As species concentrations in our study were meat, legumes and vegetables. We observed that the daily intake of 100 g of meat was associated with a 16% reduction in MMA, and when the different categories of meat were studied, only red meat remained statistically significant. This finding seems to be in agreement with the Strong Heart Family Study, where the authors observed a negative relationship between red meat consumption and ΣAs (Nigra et al., 2019). These associations may reflect nutrients found in red meat that have been associated with As metabolism, such as Zn and Fe, and certain OCM nutrients, such as vitamin B_{12}, choline and methionine (Kurzius-Spencer et al., 2017).

The consumption of legumes was also negatively associated with TAs and AB concentrations, but positively related to iAs concentrations, with an effect size similar to that of rice consumption (an increase of 30% in the iAs levels per daily intake of 100 g of any of these foods). Overall, legumes showed low levels of TAs (European Food Safety Authority, 2009), but the content of iAs was relatively high (66% of the TAs was iAs) (Agencia Catalana de Seguretat Alimentaria, 2017). In addition, Spain is the third highest consumer of legumes in the European Union (Food and Agriculture Organization of the United Nations, 2017). In our population, vegetable consumption was directly associated with ΣAs, DMA and MMA levels, each metabolite increasing by 5% per 100 g of vegetable intake. The iAs concentrations seem to vary with the type of vegetable. For example, the iAs levels measured in vegetables marketed in Valencia ranged between 0.0001 mg/kg of fresh mass in tomatoes to 0.02 mg/kg in aubergines, courgette and cucumber (Marín et al., 2018). In addition, the relatively high frequency of consumption of this type of food in our population (around 200 g per day) could explain the positive relationship with some As species.

4.3. Factors associated with As exposure: other factors

In our study, we have found differences in all As species concentrations, except for ΣAs, according to the study area. In Valencia, urinary MMA and iAs concentrations were higher than in Gipuzkoa. The dietary pattern in each area seems to be different. For example, women from Valencia consumed more rice (mean [SD] = 61 [32] grams per day) than those from Gipuzkoa (mean [SD] = 33[20] grams per day) (Table S4). Women from Valencia also consumed more shellfish and molluscs than women from Gipuzkoa. Ferrante (2019) reported that molluscs sampled on the Mediterranean coasts presented higher iAs concentrations (0.50 mg/kg wet weight, w.w.) than those sampled on the Atlantic coast (0.01 mg/kg w.w). These differences in the dietary habits could explain the geographical variability in iAs exposure observed in this study.

In our study, women born in Latin America presented lower concentrations of AB. This observation could be related to their lower consumption of fish in comparison to Spanish women (mean [SD] = 384 [210] grams per week vs. 570 [251] grams per week]). In fact, when the model was adjusted for the total fish intake, the coefficients for the women's place of origin were attenuated. Similarly, results from the US National Health and Nutrition Examination Survey showed slightly lower levels of urinary AB in a Mexican American population, compared to the Non-Hispanic white and the black population (Caldwell et al., 2009).

4.4. Evaluation of As methylation

The process underlying iAs metabolism is still not fully understood. Nowadays, the proposed iAs metabolism pathways include an enzymatic reduction (from pentavalent to trivalent forms) and a methylation process, giving rise to MMA and DMA forms, in a first and second step, respectively (Cullen, 2014). The MMA form is considered more toxic than DMA and so this methylation process is thought to be a detoxification mechanism, although it has been shown that intermediate compounds can be highly toxic (Vahter, 2002). The relative proportion of each urinary metabolite (iAs, MMA and DMA) over the sum of those species has been used to reflect the individual iAs methylation efficiency (Agency for Toxic Substances and Disease Registry, 2007). However, this method seems to be inappropriate for populations with low exposure to iAs through water and with high fish and seafood consumption, such as Spain (Navas-Acien et al., 2011), because, as a metabolite from the As species of fish, DMA could be overestimating the iAs exposure. In order to solve this problem, some strategies have been proposed, such as the residual-based method developed by Jones et al. (2016). In the present study, we have applied this approach to obtain a more accurate assessment of iAs metabolism. In fact, we have observed differences between the calibrated and non-calibrated percentages.

Several studies have used the percentages method to assess the iAs metabolism efficiency. However, a limitation of this approach is that the three percentages are highly correlated with each other, which hinders the interpretation of the results. In order to minimize this problem, some authors have proposed the use of a principal component analysis by transforming the three interrelated variables into two independent measures of As metabolism phenotypes (Balakrishnan et al., 2016; Gribble et al., 2015; Jansen et al., 2016; Spratlen et al., 2017). The first component, PC1, has been suggested to show the capacity of producing DMA (higher methylation efficiency) and explains the highest percentage of the original variance. That is, PC1 seems to represent the second step of metabolism, or the overall metabolism efficiency. In our study, PC1 agreed with these previous studies, indicating an inverse relation between %iAs and %DMA. Regarding the pattern for PC2, it has been interpreted as the first step of metabolism, or the capacity to transform iAs into MMA. In our results, PC2 showed a negative correlation between the %MMA and %iAs, regardless of %DMA. Until now, there has been no consensus on which is the most accurate approach to evaluate the efficiency of As methylation, bearing in mind that As metabolism is still under study and that the metabolites could also reflect dietary sources.

For this reason, interpretation of the results is complex and they should be taken with caution.

4.5. Factors associated with iAs methylation efficiency

One factor related to As methylation efficiency is the women's place of origin. Latin-American women presented higher methylation of iAs, represented by a higher %DMA and lower %MMA. This association remained even after adjusting for diet and other variables that can affect As exposure. This result is consistent with those of other studies, which have shown differences in metabolism depending on the ethnicity of the participants. A study carried out in USA showed a slightly higher methylation efficiency among Hispanic people than non-Hispanic white, African American and Chinese American people (Balakrishnan et al., 2018). In the same way, a recent study has reported a more efficient methylation pattern (denoted by higher %DMA and lower %iAs) in US-born and foreign-born pregnant Hispanic women, compared with their non-Hispanic counterparts (Farzan et al., 2020). Other studies found that ethnicity was the strongest factor associated with As metabolism, even if the models were adjusted for water exposure (De Loma et al., 2019; Hopenhayn-Rich et al., 1996). It has been observed that some South American populations exposed to high levels of iAs for generations have a higher iAs methylation efficiency (higher DMA excretion) that has been acquired by a higher presence of the protective genetic variants in the *Arsenic [+3 oxidation state] methyltransferase* (*AS3MT*) gene, considered to be the major contributor to As methylation efficiency (Schlebusch et al., 2015; Vahter et al., 1995).

Our results indicated a positive relationship between the women's BMI and methylation efficiency, i.e. women with higher BMI present a decrease in %iAs and %MMA and an increase in %DMA. This pattern has also been observed in previous studies (Bommarito et al., 2019; Shen et al., 2016). The mechanism underlying the relationship between As

methylation and BMI is still unclear; however, some studies postulate that worse kidney function or the intake of certain proteins, both associated with an increasing BMI, could be associated with an increased DMA excretion (Duan et al., 2019; Peters et al., 2015; Vahter, 2007).

The gestational age at sampling was inversely related to the %MMA. This result is consistent with the findings of previous studies, which show an increase in methylation efficiency throughout pregnancy, denoted by higher %DMA and lower %MMA and iAs (Gardner et al., 2011; Hopenhayn et al., 2003). It has been suggested that As metabolism is elevated during the course of pregnancy due to a more efficient maternal one-carbon metabolism that increases the endogenous synthesis of the methyl-donor choline so as to be able to supply the high fetal demand for correct development (Vahter, 2009). Nevertheless, in our study, gestational age was only related to %MMA, but it was not associated with an increase in %DMA. Unfortunately, in our study we measured As metabolite concentrations in one-spot urine samples during pregnancy, which prevents us from evaluating whether this decreasing trend in %MMA is due to an improvement in As methylation or a change in the maternal diet.

In our study, smoker women presented a lower iAs methylation efficiency by showing a decrease in PC1 (understood as the overall metabolism efficiency). This result is in agreement with previous studies (Shen et al., 2016; Tseng, 2008). Tobacco consumption has been observed to decrease concentrations of one-carbon nutrients, such as vitamin B_{12} and folate, which could be affecting the As methylation efficiency (Mouhamed et al., 2011). Another possible explanation could be related to the presence of other metals in the tobacco, such as Cd, which could modify As metabolism. This metal seems to bind to reduced glutathione (GSH) (Zalups and Ahmad, 2003), which is an antioxidant involved in the reduction from trivalent to pentavalent As species. In fact, in our population, urinary Cd concentrations were directly associated with an increase in %MMA.

We also observed an association between the estimation of Fe intake and the As metabolism efficiency, specifically with decreasing PC1. In experimental studies, this element seems to diminish the bioaccessibility of As in the gut (Yu et al., 2016). Nevertheless, in a randomized controlled trial study conducted with Mexican children, supplementation with Fe had no impact on the As metabolism (Kordas et al., 2017). In this same study, Zn supplementation was not related to better As metabolism. These results seem to be in disagreement with those found in our study, where estimated Zn consumption was positively associated with the %DMA, although the coefficients did not reach statistical significance. Similarly to our findings, in an observational study conducted on Mexican women, the estimated Zn intake was related to a decrease in %MMA and %iAs, and an increase in %DMA (López-Carrillo et al., 2016). Zn is a necessary cofactor of betaine homocysteine methyltransferase (BHMT; EC 2.1.1.5). This enzyme uses betaine as a methyl donor and Zn as a cofactor for the remethylation of homocysteine to methionine (Millian and Garrow, 1998).

Finally, we did not observe any statistically significant association between As methylation efficiency and the estimated intake of OCM nutrients. Previous studies have observed an influence of some of these nutrients on iAs methylation, such as folate and vitamins B_6 and B_{12}, which are involved in the synthesis of S-adenosylmethionine (SAM), the main donor of the methyl group in iAs methylation (Bozack et al., 2019; Gamble et al., 2005; Howe et al., 2017b; Kurzius-Spencer et al., 2017). However, the results from studies conducted on populations of pregnant women have been heterogeneous; thus, in a Mexican cohort, no association was observed between levels of vitamin B_{12} in serum and the percentages of the urinary arsenic metabolites (Laine et al., 2018). A study of pregnant women in Bangladesh showed a marginal negative association with plasma folate and %iAs, but no association with vitamin B_{12} was found (Li et al., 2008). Additionally, plasma folate was inversely associated with the urinary percentage of As^{+5} before delivery (Hall et al., 2007). However, in that same cohort, an increase in methylation efficiency throughout the pregnancy was observed regardless of the women's folate and vitamin B_{12} status (Gardner et al., 2011). It has been suggested that As metabolism is more efficient during pregnancy due to an increase in the endogenous synthesis of the methyl-donor choline, in order to meet the high fetal demand (Vahter, 2009). This specific process during pregnancy may lead to certain cofactors or methyl-donors, such as folate, having a marginal influence on As metabolism (Gardner et al., 2011). Moreover, it is possible that in well-nourished populations it is more difficult to observe the influence of the one-carbon nutrients than in populations with nutritional deficiencies. In fact, Howe (2014) revealed a positive association between blood SAM and %MMA in folate and cobalamin-deficient participants, but this association was not found in the micronutrient-sufficient group. In our populations, only 22%, 2% and 16% of women had estimated levels of vitamins B_6, B_{12} and folate intake, respectively, below the recommendations (European Food Safety Authority, 2019), and only 4% of the participants did not take folic acid supplements at the first trimester of pregnancy.

This study has several limitations: 1) around 30% of the recruited participants were not included in this study, and participants could have a more privileged socioeconomic profile than non-participants. This fact could be related to dietary habits that can lead to differences in exposure to different forms of As; 2) another limitation in our study could be related to the assessment of As exposure at only one time point during pregnancy. This could reflect only recent As exposure and it might not be representative of the period of pregnancy as a whole; 3) the present study lacks information on some important nutrients involved in OCM and related to As metabolism, such as choline and betaine, and thus the influence of these variables could not be tested; 4) finally, we used creatinine concentrations to control for urinary dilution. Creatinine concentrations seem to be associated with As metabolism. To control for this effect, we used the approach proposed by Barr et al. (2005), which involves including the creatinine concentrations in the multivariate models as a separate independent variable. Nevertheless, due to the complexity of the interrelations between As metabolism, micronutrients and creatinine, the interpretation of the factors associated with As metabolism should be taken with caution.

The major strength of the present study is the analysis of As speciation in a considerably large sample size. In fact, as far as we know, this is the largest European study describing As species concentrations and methylation efficiency in pregnant women. Another advantage is the analysis of As methylation efficiency through two approaches: using the relative percentages of each As metabolite and, in order to minimize the high correlation between the three percentages, using a principal component analysis. This allows comparability with previous studies that have used either of these two methods to evaluate the efficiency of arsenic metabolism.

5. Conclusions

The concentrations of the urinary As species in our study were slightly higher compared to other populations with low environmental exposure to As through water intake. Rice and seafood consumption, especially shellfish and molluscs, were the major contributors to the urinary concentrations of As species during pregnancy. Vegetables, legumes, eggs and other cereals contributed to the concentrations of different species of As during pregnancy. In the present study, the intake of nutrients and vitamins seemed to be weakly related to methylation efficiency. The consumption of tobacco in pregnancy, the women's place of origin and their body mass index were also associated with the methylation efficiency. Further birth cohort studies in low exposure areas are necessary to improve knowledge about prenatal arsenic exposure, especially of its inorganic forms, and its potential health impact during childhood. This information could be used to propose new strategies in public health.

Credit author statement

Raquel Soler-Blasco: Conceptualization, Methodology, Formal analysis, Writing – original draft, Writing – review & editing. Mario Murcia: Methodology, Formal analysis, Writing – review & editing. Manuel Lozano: Writing – review & editing. Blanca Sarzo: Methodology, Writing – review & editing. Ana Esplugues: Writing – review & editing. Jesús Vioque: Writing – review & editing. Nerea Lertxundi: Writing – review & editing. Loreto Santa Marina: Conceptualization, Writing – review & editing, Funding acquisition. Aitana Lertxundi: Writing – review & editing, Funding acquisition. Amaia Irizar: Methodology, Writing – review & editing. Simone Braeuer: Writing – review & editing. Walter Goesler: Writing – review & editing. Ferran Ballester: Conceptualization, Writing – original draft, Writing – review & editing, Funding acquisition. Sabrina Llop: Conceptualization, Formal analysis, Writing – original draft, Writing – review & editing, Funding acquisition.

Policy and ethics

The study protocol was approved by the Ethics Committee of the university hospital La Fe (Valencia), the Ethics Committee of the Public Health Research Centre in Valencia (CSISP) and the Ethics Committee of Donostia Hospital (Gipuzkoa). Informed consent was obtained from all participants in each phase.

Funding

This study was supported by grants from Instituto de Salud Carlos III, Spain [FIS-FEDER: 07/0314; FIS 11/01007, 13/1944, 16/1288, 19/1338; Miguel Servet- FEDER: CP15/0025, CPII20/00006; FIS-FSE: 17/00260; Miguel Servet-FSE: MS15/0025, MS20/0006; PI06/0867 and FIS-PI18/01142 incl. FEDER funds], Generalitat Valenciana, Spain BEST/2020/059, Department of Health of the Basque Government, Spain (2005111093), Provincial Government of Gipuzkoa (DFG06/002), and annual agreements with the municipalities in the study area (Zumarraga, Urretxu, Legazpi, Azkoitia, Azpeitia and Beasain).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We are grateful to all the participants and their families for taking part in this study. We also appreciate the generous contribution made to the study by all INMA members. A full roster of the INMA Project Investigators can be found at http://www.proyectoinma.org/presentacion-inma/listado-investigadores/en_listado-investigadores).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2021.110889.

References

Agencia Catalana de Seguretat Alimentaria, 2017. Contaminantes químicos. Estudio de dieta total en Cataluña, 2012. Generalitat de Catalunya. Departament de Salut., Barcelona. https://publications.iarc.fr/Book-And-Report-Series/Iarc-Monographs-On-The-Identification-Of-Carcinogenic-Hazards-To-Humans/Arsenic-Metals-Fibres-And-Dusts-2012.

Agency for Toxic Substances and Disease Registry, 2016. Addendum to the Toxicological Profile for Arsenic. Division of Toxicology and Human Health Sciences, Atlanta.

Agency for Toxic Substances and Disease Registry, 2007. Toxicological Profile for Arsenic. Division of Toxicology and Environmental Medicine, Atlanta.

Arija, V., Hernández-Martínez, C., Tous, M., Canals, J., Guxens, M., Fernández-Barrés, S., Ibarluzea, J., Izaro, B., Soler-Blasco, R., Llop, S., Vioque, J., Sunyer, J., Julvez, J., 2019. Association of iron status and intake during pregnancy with neuropsychological outcomes in children aged 7 Years: the prospective birth cohort infancia y medio ambiente (INMA) study. Nutrients 11. https://doi.org/10.3390/nu11122999.

Balakrishnan, P., Jones, M.R., Vaidya, D., Tellez-Plaza, M., Post, W.S., Kaufman, J.D., Bielinski, S.J., Taylor, K., Francesconi, K., Goessler, W., Navas-Acien, A., 2018. Ethnic, geographic, and genetic differences in arsenic metabolism at low arsenic exposure: a preliminary analysis in the multi-ethnic study of atherosclerosis (MESA). Int. J. Environ. Res. Publ. Health 15, 1–11. https://doi.org/10.3390/ijerph15061179.

Balakrishnan, P., Vaidya, D., Franceschini, N., Voruganti, V.S., Gribble, M.O., Haack, K., Laston, S., Umans, J.G., Francesconi, K.A., Goessler, W., North, K.E., Lee, E., Yracheta, J., Best, L.G., MacCluer, J.W., Kent, J., Cole, S.A., Navas-Acien, A., 2016. Association of cardiometabolic genes with arsenic metabolism biomarkers in American Indian communities: the Strong Heart family study (SHFS). Environ. Health Perspect. 125, 15–22. https://doi.org/10.1289/EHP251.

Barr, D.B., Wilder, L.C., Caudill, S.P., Gonzalez, A.J., Needham, L.L., Pirkle, J.L., 2005. Urinary creatinine concentrations in the U.S. population: implications for urinary biologic monitoring measurements. Environ. Health Perspect. 113, 192–200. https://doi.org/10.1289/EHP.7337.

Bommarito, P.A., Xu, X., González-Horta, C., Sánchez-Ramirez, B., Ballinas-Casarrubias, L., Luna, R.S., Pérez, S.R., Hernández Ávila, J.E., García-Vargas, G.G., Del Razo, L.M., Stýblo, M., Mendez, M.A., Fry, R.C., 2019. One-carbon metabolism nutrient intake and the association between body mass index and urinary arsenic metabolites in adults in the Chihuahua cohort. Environ. Int. 123, 292–300. https://doi.org/10.1016/j.envint.2018.12.004.

Bornhorst, J., Ebert, F., Meyer, S., Ziemann, V., Xiong, C., Guttenberger, N., Raab, A., Baesler, J., Aschner, M., Feldmann, J., Francesconi, K., Raber, G., Schwerdtle, T., 2020. Toxicity of three types of arsenolipids: species-specific effects in Caenorhabditis elegans. Metall 12, 794–798. https://doi.org/10.1039/d0mt00039f.

Bozack, A.K., Hall, M.N., Liu, X., Ilievski, V., Lomax-luu, A.M., Parvez, F., Siddique, A.B., 2019. Folic acid supplementation enhances arsenic methylation : results from a folic acid and creatine supplementation randomized controlled trial in Bangladesh. Am. J. Clin. Nutr. 109, 380–391. https://doi.org/10.1093/ajcn/nqy148.

Caldwell, K.L., Jones, R.L., Verdon, C.P., Jarrett, J.M., Caudill, S.P., Osterloh, J.D., 2009. Levels of urinary total and speciated arsenic in the US population: national health and nutrition examination Survey 2003-2004. J. Expo. Sci. Environ. Epidemiol. 19, 59–68. https://doi.org/10.1038/jes.2008.32.

Challenger, F., 1945. Biological methylation. Chem. Rev. 36, 315–361. https://doi.org/10.1021/cr60115a003.

Chou, W.C., Chung, Y.T., Chen, H.Y., Wang, C.J., Ying, T.H., Chuang, C.Y., Tseng, Y.C., Wang, S.L., 2014. Maternal arsenic exposure and DNA damage biomarkers, and the associations with birth outcomes in a general population from Taiwan. PloS One 9, e86398. https://doi.org/10.1371/journal.pone.0086398.

Cullen, W.R., 2014. Chemical mechanism of arsenic biomethylation. Chem. Res. Toxicol. 27, 457–461. https://doi.org/10.1021/tx400441h.

De Loma, J., Tirado, N., Ascui, F., Levi, M., Vahter, M., Broberg, K., Gardon, J., 2019. Elevated arsenic exposure and efficient arsenic metabolism in indigenous women around Lake Poopó Bolivia. Sci. Total Environ. 657, 179–186. https://doi.org/10.1016/j.scitotenv.2018.11.473.

Drobná, Z., Walton, F.S., Paul, D.S., Xing, W., Thomas, D.J., Stýblo, M., 2010. Metabolism of arsenic in human liver: the role of membrane transporters. Arch. Toxicol. 84, 3–16. https://doi.org/10.1007/s00204-009-0499-7.

Duan, Y., Wang, X., Zhang, J., Ye, P., Cao, R., Yang, X., Bai, Y., Wu, H., 2019. Body mass index is an independent predictive factor for kidney function evaluated by glomerular filtration rate in a community-dwelling population. Eat. Weight Disord 24, 731–738. https://doi.org/10.1007/s40519-017-0434-5.

Ettinger, A.S., Arbuckle, T.E., Fisher, M., Liang, C.L., Davis, K., Cirtiu, C.M., Bélanger, P., LeBlanc, A., Fraser, W.D., 2017. Arsenic levels among pregnant women and newborns in Canada: results from the Maternal-Infant Research on Environmental Chemicals (MIREC) cohort. Environ. Res. 153, 8–16. https://doi.org/10.1016/j.envres.2016.11.008.

European Commission, 2015. Commission Regulation (EU) 2015/1006 of 25 June 2015 Amending Regulation (EC) No 1881/2006 as Regards Maximum Levels of Inorganic Arsenic in Foodstuffs.

European Food Safety Authority, 2019. Dietary Reference Values for the EU [WWW Document]. DVR Finder. URL. https://www.efsa.europa.eu/en/interactive-pages/drvs.

European Food Safety Authority, 2014. Dietary exposure to inorganic arsenic in the European population. Eur. Food Saf. Auth. J. 12 https://doi.org/10.2903/j.efsa.2014.3597.

European Food Safety Authority, 2009. Scientific opinion on arsenic in food. Eur. Food Saf. Auth. J. 7 https://doi.org/10.2903/j.efsa.2009.1351.

Farzan, S.F., Howe, C.G., Chavez, T.A., Hodes, T.L., Johnston, J.E., Habre, R., Dunton, G., Bastain, T.M., Breton, C.V., 2020. Demographic predictors of urinary arsenic in a low-income predominantly Hispanic pregnancy cohort in Los Angeles. J. Expo. Sci. Environ. Epidemiol. https://doi.org/10.1038/s41370-020-0251-1.

Ferrante, M., Napoli, S., Zuccarello, P., Cristaldi, A., 2019. Systematic review of arsenic in fresh seafood from the Mediterranean Sea and European Atlantic coasts : a health risk assessment. Food Chem. Toxicol. 126, 322–331. https://doi.org/10.1016/j.fct.2019.01.010.

Food and Agriculture Organization of the United Nations, 2017. Food Balance Sheets [WWW Document]. FAOSTAT.

Fowler, B.A., Chou, S.C., Jones, R.L., Sullivan, D.W., Chen, C., 2015. Arsenic. In: Nordberg, G., Fowler, B., Nordberg, M. (Eds.), Handbook on the Toxicology of Metals. Elsevier, pp. 581–624. https://doi.org/10.1016/B978-0-444-59453-2.00028-7.

Freire, C., Amaya, E., Gil, F., Fernández, M.F., Murcia, M., Llop, S., Andiarena, A., Aurrekoetxea, J., Bustamante, M., Guxens, M., Ezama, E., Fernández-Tardón, G., Olea, N., 2018. Prenatal co-exposure to neurotoxic metals and neurodevelopment in preschool children: the Environment and Childhood (INMA) Project. Sci. Total Environ. 621, 340–351. https://doi.org/10.1016/j.scitotenv.2017.11.273.

Gamble, M.V., Liu, X., Ahsan, H., Pilsner, J.R., Ilievski, V., Slavkovich, V., Parvez, F., Chen, Y., Levy, D., Factor-Litvak, P., Graziano, J.H., 2006. Folate and arsenic metabolism: a double-blind, placebo-controlled folic acid-supplementation trial in Bangladesh. Am. J. Clin. Nutr. 84, 1093–1101. https://doi.org/10.1093/ajcn/84.5.1093.

Gamble, M.V., Liu, X., Ahsan, H., Pilsner, J.R., Illievski, V., Slavkovich, V., Parvez, F., Levy, D., Factor-Litvak, P., Graziano, J.H., 2005. Folate, homocysteine, and arsenic metabolism in arsenic-exposed individuals in Bangladesh. Environ. Health Perspect. 113, 1683–1688. https://doi.org/10.1289/ehp.8084.

Gamboa-loira, B., Cebrián, M.E., Franco-marina, F., López-carrillo, L., 2017. Arsenic metabolism and cancer risk : a meta-analysis. Environ. Res. 156, 551–558. https://doi.org/10.1016/j.envres.2017.04.016.

Gao, S., Lin, P., Mostofa, G., Quamruzzaman, Q., Rahman, M., Rahman, M.L., Su, L., Hsueh, Y., Weisskopf, M., Coull, B., Christiani, D.C., 2019. Determinants of arsenic methylation efficiency and urinary arsenic level in pregnant women in Bangladesh. Environ. Health Global Access Sci. Source 18.

Gardner, R.M., Nermell, B., Kippler, M., Grandér, M., Li, L., Ekström, E.C., Rahman, A., Lönnerdal, B., Hoque, A.M.W., Vahter, M., 2011. Arsenic methylation efficiency increases during the first trimester of pregnancy independent of folate status. Reprod. Toxicol. 31, 210–218. https://doi.org/10.1016/j.reprotox.2010.11.002.

Gilbert-Diamond, D., Cottingham, K.L., Gruber, J.F., Punshon, T., Sayarath, V., Gandolfi, A.J., Baker, E.R., Jackson, B.P., Folt, C.L., Karagas, M.R., 2011. Rice consumption contributes to arsenic exposure in US women. Proc. Natl. Acad. Sci. Unit. States Am. 108, 20656–20660. https://doi.org/10.1073/pnas.1109127108.

Gossai, A., Lesseur, C., Farzan, S., Marsit, C., Karagas, M.R., Gilbert-Diamond, D., 2015. Association between maternal urinary arsenic species and infant cord blood leptin levels in a New Hampshire Pregnancy Cohort. Environ. Res. 136, 180–186. https://doi.org/10.1016/J.ENVRES.2014.10.005.

Gribble, M.O., Voruganti, V.S., Cole, S.A., Haack, K., Balakrishnan, P., Laston, S.L., Tellez-Plaza, M., Francesconi, K.A., Goessler, W., Umans, J.G., Thomas, D.C., Gilliland, F., North, K.E., Franceschini, N., Navas-Acien, A., 2015. Linkage analysis of urine arsenic species patterns in the Strong Heart family study. Toxicol. Sci. 148, 89–100. https://doi.org/10.1093/toxsci/kfv164.

Guxens, M., Ballester, F., Espada, M., Ferna, M.F., Grimalt, J.O., Ibarluzea, J., Olea, N., Rebagliato, M., Tardón, A., Torrent, M., Vioque, J., Vrijheis, M., Sunyer, J., INMA project, 2012. Cohort Profile : the INMA — INfancia y medio ambiente —(environment and childhood) project. Int. J. Epidemiol. 41, 930–940. https://doi.org/10.1093/ije/dyr054.

Hall, M., Gamble, M., Slavkovich, V., Liu, X., Levy, D., Cheng, Z., van Geen, A., Yunus, M., Rahman, M., Pilsner, J.R., Graziano, J., 2007. Determinants of arsenic metabolism: blood arsenic metabolites, plasma folate, cobalamin, and homocysteine concentrations in maternal-newborn pairs. Environ. Health Perspect. 115, 1503–1509. https://doi.org/10.1289/ehp.9906.

Hamadani, J., Tofail, F., Nermell, B., Gardner, R., Shiraji, S., Bottai, M., Arifeen, S., Huda, S., Vahter, M., 2011. Critical windows of exposure for arsenic-associated impairment of cognitive function in pre-school girls and boys: a population-based cohort study. Int. J. Epidemiol. 40, 1593–1604. https://doi.org/10.1093/ije/dyr176.

Heck, J.E., Gamble, M.V., Chen, Y., Graziano, J.H., Slavkovich, V., Parvez, F., Baron, J. A., Howe, G.R., Ahsan, H., 2007. Consumption of folate-related nutrients and metabolism of arsenic in Bangladesh. Am. J. Clin. Nutr. 85, 1367–1374. https://doi.org/10.1093/ajcn/85.5.1367.

Hopenhayn-Rich, C., Biggs, M. Lou, Smith, A.H., Kalman, D.A., Moore, L.E., 1996. Methylation study of a population environmentally exposed to arsenic in drinking water. Environ. Health Perspect. 104, 620. https://doi.org/10.2307/3433091.

Hopenhayn, C., Huang, B., Christian, J., Peralta, C., Ferreccio, C., Atallah, R., Kalman, D., 2003. Profile of urinary arsenic metabolites during pregnancy. Environ. Health Perspect. 111, 1888–1891. https://doi.org/10.1289/ehp.6254.

Howe, C.G., Farzan, S.F., Garcia, E., Jursa, T., Iyer, R., Berhane, K., Chavez, T.A., Hodes, T.L., Grubbs, B.H., Funk, W.E., Smith, D.R., Bastain, T.M., Breton, C.V., 2020. Arsenic and birth outcomes in a predominately lower income Hispanic pregnancy cohort in Los Angeles. Environ. Res. 184, 109294. https://doi.org/10.1016/j.envres.2020.109294.

Howe, C.G., Li, Z., Zens, M.S., Palys, T., Chen, Y., Channon, J.Y., Karagas, M.R., Farzan, S.F., 2017a. Dietary B vitamin intake is associated with lower urinary monomethyl arsenic and oxidative stress marker 15-F2t-isoprostane among New Hampshire adults. J. Nutr. 147, 2289–2296. https://doi.org/10.3945/jn.117.253419.

Howe, C.G., Li, Z., Zens, M.S., Palys, T., Chen, Y., Channon, J.Y., Karagas, M.R., Farzan, S.F., 2017b. Dietary B vitamin intake is associated with lower urinary monomethyl arsenic and oxidative stress marker 15-F2t-isoprostane among New Hampshire adults. J. Nutr. 147, 2289–2296. https://doi.org/10.3945/jn.117.253419.

Howe, C.G., Niedzwiecki, M.M., Hall, M.N., Liu, X., Ilievski, V., Slavkovich, V., Alam, S., Siddique, A.B., Graziano, J.H., Gamble, M.V., 2014. Folate and cobalamin modify associations between S-adenosylmethionine and methylated arsenic metabolites in arsenic-exposed bangladeshi adults1-3. J. Nutr. 144, 690–697. https://doi.org/10.3945/jn.113.188789.

International Agency for Research on Cancer, 2012. Arsenic, Metals, Fibres and dusts. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Iarc Monographs.

Jansen, R.J., Argos, M., Tong, L., Li, J., Rakibuz-Zaman, Muammad Islam, T., Slavkovich, V., Ahmed, A., Navas-Acien, A., Parvez, F., Yu, C., Mary, G., Graziano, J. H., Pierce, B.L., Ahsan, H., 2016. Determinants and consequences of arsenic metabolism efficiency among 4,794 individuals: demographics, lifestyle, genetics, and toxicity. Cancer Epidemiol. biomarkers Prev. 25, 381–390. https://doi.org/10.1016/j.cogdev.2010.08.003.Personal.

Jay Christian, W., Hopenhayn, C., Centeno, J.A., Todorov, T., 2006. Distribution of urinary selenium and arsenic among pregnant women exposed to arsenic in drinking water. Environ. Res. 100, 115–122. https://doi.org/10.1016/j.envres.2005.03.009.

Jones, M.R., Tellez-Plaza, M., Vaidya, D., Grau, M., Francesconi, K.A., Goessler, W., Guallar, E., Post, W.S., Kaufman, J.D., Navas-Acien, A., 2016. Estimation of inorganic arsenic exposure in populations with frequent seafood intake: evidence from MESA and NHANES. Am. J. Epidemiol. 184, 590–602. https://doi.org/10.1093/aje/kww097.

Kordas, K., Roy, A., López, P., García-Vargas, G., Cebrián, M.E., Vera-Aguilar, E., Rosado, J.L., 2017. Iron and zinc supplementation does not impact urinary arsenic excretion in Mexican school children. J. Pediatr. 185, 205–210. https://doi.org/10.1016/j.jpeds.2017.02.040.

Kurzius-Spencer, M., Thomson, C.A., Hartz, V., Hsu, C., Burgess, J.L., Kay, M., Rourke, O., Harris, R.B., 2017. Nutrients in one-carbon metabolism and urinary arsenic methylation in the national health and nutrition examination Survey (NHANES) 2003–2004. Sci. Total Environ. 607– 608, 381–390. https://doi.org/10.1016/j.scitotenv.2017.07.019.

Laine, J.E., Bailey, K.A., Rubio-andrade, M., Olshan, A.F., Smeester, L., 2015. Maternal arsenic exposure , arsenic methylation efficiency , and birth outcomes in the biomarkers of exposure to ARsenic (BEAR) pregnancy cohort in Mexico. Child. Heal. 123, 186–192.

Laine, J.E., Ilievski, V., Richardson, D.B., Herring, A.H., Stýblo, M., Rubio-Andrade, M., Garcia-Vargas, G., Gamble, M.V., Fry, R.C., 2018. Maternal one carbon metabolism and arsenic methylation in a pregnancy cohort in Mexico. J. Expo. Sci. Environ. Epidemiol. 28, 505–514. https://doi.org/10.1038/s41370-018-0041-1.

Li, L., Ekström, E.C., Goessler, W., Lönnerdal, B., Nermell, B., Yunus, M., Rahman, A., El Arifeen, S., Persson, L.Å., Vahter, M., 2008. Nutritional status has marginal influence on the metabolism of inorganic arsenic in pregnant Bangladeshi women. Environ. Health Perspect. 116, 315–321. https://doi.org/10.1289/ehp.10639.

Litter, M.I., Armienta, M.A., Villanueva Estrada, R.E., Villaamil Lepori, E.C., Olmos, V., 2020. Arsenic in Latin-America: Part I. In: Srivastava, S. (Ed.), Arsenic in Drinking Water and Food. Springer Nature Singapore, Singapore.

López-Carrillo, L., Gamboa-Loira, B., Becerra, W., Hernández-Alcaraz, C., Hernández-Ramírez, R., Gandolfi, A., Cebrián, M., 2016. Dietary micronutrient intake and its relationship with arsenic metabolism in Mexican women. Environ. Res. 151, 445–450. https://doi.org/10.1016/j.envres.2016.08.015.Dietary.

Lozano, M., Murcia, M., Soler-Blasco, R., Iñiguez, C., Irizar, A., Lertxundi, A., Basterrechea, M., Marina, L.S., Amorós, R., Broberg, K., Ballester, F., Llop, S., 2020. Prenatal Se concentrations and anthropometry at birth in the INMA study (Spain). Environ. Res. 108943. https://doi.org/10.1016/j.envres.2019.108943.

Marín, S., Pardo, O., Sanchez, A., Sanchis, Y., Vélez, D., Devesa, V., Font, G., Yusà, V., 2018. Assessment of metal levels in foodstuffs from the region of Valencia region (Spain). Toxicol. Reports 5, 654–670. https://doi.org/10.1016/J.TOXREP.2018.05.005.

Millian, N.S., Garrow, T.A., 1998. Human betaine-homocysteine methyltransferase is a zinc metalloenzyme. Arch. Biochem. Biophys. 356, 93–98. https://doi.org/10.1006/abbi.1998.0757.

Molin, M., Ulven, S., Dahl, L., Telle-Hansen, V., Holck, M., Skjegstad, G., Ledsaak, O., Sloth, J.J., Goessler, W., Oshaug, A., Alexander, J., Fliegel, D., Ydersbond, T.A., Meltzer, H.M., 2012. Humans seem to produce arsenobetaine and dimethylarsinate after a bolus dose of seafood. Environ. Res. 112, 28–39. https://doi.org/10.1016/j.envres.2011.11.007.

Moon, K., Guallar, E., Navas-Acien, A., 2012. Arsenic exposure and cardiovascular disease: an updated systematic review. Curr. Atheroscler. reports. 14, 542–555. https://doi.org/10.1007/s11883-012-0280-x.

Mouhamed, D.H., Ezzaher, A., Neffati, F., Douki, W., Najjar, M.F., 2011. Effect of cigarette smoking on plasma homocysteine concentrations. Clin. Chem. Lab. Med. 49, 479–483. https://doi.org/10.1515/CCLM.2011.062.

Navas-Acien, A., Francesconi, K.A., Silbergeld, E.K., Guallar, E., 2011. Seafood intake and urine concentrations of total arsenic, dimethylarsinate and arsenobetaine in the US population. Environ. Res. 111, 110–118. https://doi.org/10.1016/j.envres.2010.10.009.

Neamtiu, I., Bloom, M.S., Gati, G., Goessler, W., Surdu, S., Pop, C., Braeuer, S., Fitzgerald, E.F., Baciu, C., Lupsa, I.R., Anastasiu, D., Gurzau, E., 2015. Pregnant women in timis county, Romania are exposed primarily to low-level (<10μg/l) arsenic through residential drinking water consumption. Int. J. Hyg Environ. Health 218, 371–379. https://doi.org/10.1016/j.ijheh.2015.01.004.

Nigra, A.E., Olmedo, P., Grau-perez, M., Leary, R.O., Leary, M.O., Fretts, A.M., Umans, J. G., Best, L.G., Francesconi, K.A., Goessler, W., Cole, S.A., Navas-acien, A., 2019. Dietary determinants of inorganic arsenic exposure in the Strong Heart family study. Environ. Res. 177, 108616. https://doi.org/10.1016/j.envres.2019.108616.

Nordberg, G.F., Jin, T., Hong, F., Zhang, A., Buchet, J.P., Bernard, A., 2005. Biomarkers of cadmium and arsenic interactions. Toxicol. Appl. Pharmacol. 206, 191–197. https://doi.org/10.1016/J.TAAP.2004.11.028.

Palma, I., Farran, P., Cervera, P., 2008. Tables of Food Composition for Household Measures of Habitual Consumption in Spain: CESNID, fourth ed. McGraw Hill, Madrid.

Parajuli, R.P., Fujiwara, T., Umezaki, M., Watanabe, C., 2013. Association of cord blood levels of lead, arsenic, and zinc with neurodevelopmental indicators in newborns: a birth cohort study in Chitwan Valley, Nepal. Environ. Res. 121, 45–51. https://doi.org/10.1016/J.ENVRES.2012.10.010.

Peters, B.A., Hall, M.N., Liu, X., Slavkovich, V., Ilievski, V., Alam, S., Siddique, A.B., Islam, T., Graziano, J.H., Gamble, M.V., 2015. Renal function is associated with indicators of arsenic methylation capacity in Bangladeshi adults. Environ. Res. 143, 123–130. https://doi.org/10.1016/j.envres.2015.10.001.

Quansah, R., Armah, F.A., Essumang, D.K., Luginaah, I., Clarke, E., 2015. Association of arsenic with adverse pregnancy outcomes/infant. Mortality :A Systematic Review and Meta-Analysis 412, 412–421.

R Core Team, 2017. R: A Language and Environment for Statistical Computing.

Rahman, M.M., Hossain, K.F.B., Banik, S., Sikder, M.T., Akter, M., Bondad, S.E.C., Rahaman, M.S., Hosokawa, T., Saito, T., Kurasaki, M., 2019. Selenium and zinc protections against metal-(loids)-induced toxicity and disease manifestations: a review. Ecotoxicol. Environ. Saf. 168, 146–163. https://doi.org/10.1016/j.ecoenv.2018.10.054.

Sanchez, T.R., Perzanowski, M., Graziano, J.H., 2016. Inorganic arsenic and respiratory health, from early life exposure to sex-specific effects: a systematic review. Environ. Res. 147, 537–555. https://doi.org/10.1016/j.envres.2016.02.009.

Saxena, R., Bozack, A., Gamble, M.V., 2018. Nutritional influences on one-carbon metabolism: effects on arsenic methylation and toxicity. Annu. Rev. Nutr. 21, 401–429. https://doi.org/10.1146/annurev-nutr-082117-051757. Nutritional.

Scheer, J., Findenig, S., Goessler, W., Francesconi, K.A., Howard, B., G, U.J., Pollak, J., Tellez-Plaza, M., Silbergeld, E.K., Guallar, E., Navas-Acien, A., 2012. Arsenic species and selected metals in human urine: validation of HPLC/ICPMS and ICPMS procedures for a long-term population-based epidemiological study. Anal Methods 4, 406–413. https://doi.org/10.1039/C2AY05638K.

Schlebusch, C.M., Gattepaille, L.M., Engström, K., Vahter, M., Jakobsson, M., Broberg, K., 2015. Human adaptation to arsenic-rich environments. Mol. Biol. Evol. 32, 1544–1555. https://doi.org/10.1093/molbev/msv046.

Shen, H., Niu, Q., Xu, M., Rui, D., Xu, S., Feng, G., 2016. Factors affecting arsenic methylation in arsenic-exposed Humans : a systematic review and meta-analysis. Int. J. Environ. Res. Publ. Health 13. https://doi.org/10.3390/ijerph13020205.

Signes-Pastor, A.J., Carey, M., Carbonell-Barrachina, A.A., Moreno-Jiménez, E., Green, A.J., Meharg, A.A., 2016. Geographical variation in inorganic arsenic in paddy field samples and commercial rice from the Iberian Peninsula. Food Chem. 202, 356–363. https://doi.org/10.1016/j.foodchem.2016.01.117.

Soler-Blasco, R., Murcia, M., Lozano, M., González-Safont, L., Amorós, R., Ibarluzea, J., Broberg, K., Irizar, A., Lopez-Espinosa, M., Lertxundi, N., Santa Marina, L., Ballester, F., Llop, S., 2020. Prenatal manganese exposure and neuropsychological development in early childhood in the INMA cohort. Int. J. Hyg Environ. Health 224, 113443. https://doi.org/10.1016/j.ijheh.2019.113443.

Spratlen, M.J., Gamble, M.V., Grau-Perez, Ma, Chin-Chi, K., Best, L.G., Yracheta, J., Francesconi, K., Goessler, W., Mossavar-Rahmani, Y., Hall, M., Umans, J.G., Fretts, A., Navas-Acien, A., 2017. Arsenic metabolism and one-carbon metabolism at low- moderate arsenic exposure: evidence from the Strong Heart Study. Food Chem. Toxicol. 105, 387–397. https://doi.org/10.1016/j.fct.2017.05.004.Arsenic.

Stajnko, A., Šlejkovec, Z., Mazej, D., France-štiglic, A., Sešek, A., Prpić, I., Špirić, Z., Horvat, M., Falnoga, I., 2019. INMT , and MT2A polymorphisms in Croatian-Slovenian population from PHIME-CROME study. Environ. Res. 170, 301–319. https://doi.org/10.1016/j.envres.2018.11.045.

Sun, H., Rathinasabapathi, B., Wu, B., Luo, J., Pu, L., Ma, L.Q., 2014. Arsenic and selenium toxicity and their interactive effects in humans. Environ. Int. 69, 148–158. https://doi.org/10.1016/j.envint.2014.04.019.

Taylor, V.F., Li, Z., Sayarath, V., Palys, T.J., Morse, K.R., Scholz-Bright, R.A., Karagas, M. R., 2017a. Distinct arsenic metabolites following seaweed consumption in humans. Sci. Rep. 7, 1–9. https://doi.org/10.1038/s41598-017-03883-7.

Taylor, V., Goodale, B., Raab, A., Schwerdtle, T., Reimer, K., Conklin, S., Karagas, M.R., Francesconi, K.A., 2017b. Human exposure to organic arsenic species from seafood. Sci. Total Environ. 15, 266–282. https://doi.org/10.1016/j.scitotenv.2016.12.113.Human.

Tseng, C.-H., 2008. A review on environmental factors regulating arsenic methylation in humans. Toxicol. Appl. Pharmacol. 235, 338–350. https://doi.org/10.1016/j.taap.2008.12.016.

Tseng, C.H., 2007. Arsenic methylation, urinary arsenic metabolites and human diseases: current perspective. J. Environ. Sci. Health Part C Environ. Carcinog. Ecotoxicol. Rev. 25, 1–22. https://doi.org/10.1080/10590500701201695.

U.S., 2007. Department of Agriculture: Agricultural Research Service USDA. National Nutrient Database for Standard Reference. Release 25. [WWW Document]. URL. https://www.ars.usda.gov/ARSUserFiles/80400525/Data/SR25/sr25_doc.pdf.

Vahter, M., 2009. Effects of arsenic on maternal and fetal health. Annu. Rev. Nutr. 29, 381–399. https://doi.org/10.1146/annurev-nutr-080508-141102.

Vahter, M., 2002. Mechanisms of arsenic biotransformation. Toxicology 181–182, 211–217. https://doi.org/10.1016/S0300-483X(02)00285-8.

Vahter, M., Concha, G., Nermell, B., Nilsson, R., Dulout, F., Natarajan, A.T., 1995. A unique metabolism of inorganic arsenic in native Andean women. Eur. J. Pharmacol. Environ. Toxicol. 293, 455–462. https://doi.org/10.1016/0926-6917(95)90066-7.

Vahter, M.E., 2007. Interactions between arsenic-induced toxicity and nutrition in early life. J. Nutr. 137, 2798–2804. https://doi.org/10.1093/jn/137.12.2798.

Vahter, M.E., 1999. Methylation of inorganic arsenic in different mammalian species and population groups. Sci. Prog. 82, 69–88. https://doi.org/10.1177/003685049908200104.

Valeri, L., Mazumdar, M.M., Bobb, J.F., Henn, B.C., Rodrigues, E., Sharif, O.I.A., Kile, M. L., Quamruzzaman, Q., Afroz, S., Golam, M., Amarasiriwardena, C., Bellinger, D.C., Christiani, D.C., Coull, B.A., Wright, R.O., 2016. The joint effect of prenatal exposure to metal mixtures on neurodevelopmental outcomes at 20 – 40 Months of Age : evidence from rural Bangladesh. Environ. Health 125, 067015. https://doi.org/10.1289/EHP614.

Vaughan Watson, C., Lewin, M., Ragin-Wilson, A., Jones, R., Jarrett, J.M., Wallon, K., Ward, C., Hilliard, N., Irvin-barnwell, E., 2020. Characterization of trace elements exposure in pregnant women in the United States, NHANES 1999–2016. Environ. Res. 183, 109208. https://doi.org/10.1016/j.envres.2020.109208.

Vioque, J., Navarrete-Muñoz, E.-M., Gimenez-Monzó, D., García-de-la-Hera, M., Granado, F., Young, I.S., Ramón, R., Ballester, F., Murcia, M., Rebagliato, M., Iñiguez, C., 2013. Reproducibility and validity of a food frequency questionnaire among pregnant women in a Mediterranean area. Nutr. J. 12, 26. https://doi.org/10.1186/1475-2891-12-26.

Willet, W., 2013. Nutritional Epidemiology. Oxford University Press, Oxford.

World Health Organization, 2017. Guidelines for Drinking-Water Quality: Fourth Edition Incorporating the First Addendum. World Health Organization, Geneva.

Yu, H., Wu, B., Zhang, X.X., Liu, S., Yu, J., Cheng, S., Ren, H.Q., Ye, L., 2016. Arsenic metabolism and toxicity influenced by ferric iron in simulated gastrointestinal tract and the roles of gut microbiota. Environ. Sci. Technol. 50, 7189–7197. https://doi.org/10.1021/acs.est.6b01533.

Zalups, R.K., Ahmad, S., 2003. Molecular handling of cadmium in transporting epithelia. Toxicol. Appl. Pharmacol. 186, 163–188. https://doi.org/10.1016/S0041-008X(02)00021-2.

5.3 ARTÍCULO III

Soler-Blasco, R., Murcia, M., Lozano, M., Sarzo, B., Esplugues, A., Riutort-mayol, G., Vioque, J., Lertxundi, N., Santa, L., Lertxundi, A., Irizar, A., Braeuer, S., Ballester, F., & Llop, S. (2022). Prenatal arsenic exposure, arsenic methylation efficiency, and neuropsychological development among preschool children in a Spanish birth cohort. Environmental Research, 207, 112208.

Disponible en: https://doi.org/10.1016/j.envres.2021.112208

Resumen

Introducción: La exposición prenatal a arsénico (As) podría afectar negativamente el desarrollo neuropsicológico en la infancia, pero la evidencia actual es no concluyente. **Objetivos:** Explorar la relación entre las concentraciones urinarias prenatales de As total (TAs), las especies As y la eficiencia de metilación, y el desarrollo neuropsicológico infantil en una cohorte de nacimiento española. También se estudió la modificación de efecto producida por sexo del niño, así como varios nutrientes y elementos. **Materiales y Métodos:** Los sujetos de estudio fueron 807 parejas madre-hijo participantes del Proyecto INMA (Infancia y Medio Ambiente). Las concentraciones urinarias de TAs y sus metabolitos ácido monometilarsónico (MMA), ácido dimetilarsínico (DMA), As inorgánicos (iAs) y arsenobetaína se midieron en el primer trimestre del embarazo. La eficiencia de metilación se determinó a través de los porcentajes de los metabolitos y utilizando el análisis de componentes principales. El desarrollo neuropsicológico de los niños y niñas se evaluó a los 4- 5 años de edad utilizando las Escalas de Habilidades Infantiles de McCarthy (MSCA). Se construyeron modelos de regresión lineal multivariable para evaluar la asociación entre TAs, las especies As y la eficiencia de metilación materna, y las puntuaciones neuropsicológicas. Exploramos la modificación del efecto por sexo, niveles de ferritina, niveles de nutrientes maternos (manganeso y selenio séricos y zinc urinario) y la ingesta de vitaminas de la madre (folato y vitaminas B_{12} y B_6). **Resultados:** La media geométrica e intervalo de confianza al 95% (IC del 95%) de ∑As (suma de DMA, MMA e iAs) fue de 7,78 (7,41-8,17) µg/g de creatinina. Las concentraciones de MMA se asociaron inversamente con las puntuaciones de las escalas general, verbal, cuantitativa, de memoria, de función ejecutiva y de memoria de trabajo (β [IC95%] = −1,37 [−2,33 a −0,41] para la escala general). Se encontró una asociación inversa entre %MMA y las puntuaciones en la escala de memoria. Los niños y niñas cuyas madres tenían concentraciones más bajas de manganeso, zinc y ferritina obtuvieron puntuaciones más bajas en varias escalas de MSCA con una eficiencia de metilación decreciente. **Conclusión:** Se observó una asociación inversa entre las concentraciones de MMA y el desarrollo neuropsicológico a los 4-5 años de edad. Los niveles maternos de manganeso, zinc y ferritina afectaron la asociación entre la eficiencia de metilación del As y las puntuaciones de MSCA.

Environmental Research 207 (2022) 112208

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Prenatal arsenic exposure, arsenic methylation efficiency, and neuropsychological development among preschool children in a Spanish birth cohort

Raquel Soler-Blasco [a], Mario Murcia [a,b], Manuel Lozano [a,c], Blanca Sarzo [d], Ana Esplugues [a,e,f], Gabriel Riutort-Mayol [d], Jesús Vioque [g], Nerea Lertxundi [h,i], Loreto Santa Marina [e,h,j], Aitana Lertxundi [e,h,k,*], Amaia Irizar [h,k], Simone Braeuer [l], Ferran Ballester [a,e,f], Sabrina Llop [a,e]

[a] Epidemiology and Environmental Health Joint Research Unit, FISABIO–Universitat Jaume I–Universitat de València, Valencia, Spain
[b] Health Information Systems Analysis Service, Conselleria de Sanitat, Generalitat Valenciana, Valencia, Spain
[c] Preventive Medicine and Public Health, Food Sciences, Toxicology and Forensic Medicine Department, Universitat de València, Valencia, Spain
[d] Foundation for the Promotion of Health and Biomedical Research in the Valencian Region, FISABIO-Public Health, Valencia, Spain
[e] Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Madrid, Spain
[f] Department of Nursing, Universitat de València, Valencia, Spain
[g] Alicante Institute for Health and Biomedical Research, ISABIAL-UMH, Alicante, Spain
[h] Biodonostia Health Research Institute, San Sebastian, Spain
[i] Faculty of Psychology of the University of the Basque Country, UPV/ EHU, San Sebastian, Spain
[j] Public Health Division of Gipuzkoa, Basque Government, San Sebastian, Spain
[k] Departament of Preventive Medicine and Public Health of the University of the Basque Country, UPV/EHU, Leioa, Spain
[l] Institute of Chemistry, University of Graz, Graz, Austria

ARTICLE INFO

Keywords:
Arsenic
Methylation
Prenatal exposure
Neurodevelopment
Spain

ABSTRACT

Background: Prenatal arsenic (As) exposure could negatively affect child neuropsychological development, but the current evidence is inconclusive.
Objectives: To explore the relationship between prenatal urinary total As (TAs) concentrations, the As species and the methylation efficiency, and child neuropsychological development in a Spanish birth cohort. We also studied the effect modification produced by sex and several nutrients and elements.
Materials and methods: Study subjects were 807 mother–child pairs participating in the INMA (Childhood and Environment) Project. Urinary TAs and its metabolites, monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), inorganic As (iAs) and arsenobetaine were measured in the first trimester of pregnancy. Methylation efficiency was determined through the percentages of the metabolites and using principal component analysis. Children's neuropsychological development was assessed at the age of 4–5 years using the McCarthy Scales of Children's Abilities (MSCA). Multivariable linear regression models were built to assess the association between TAs, the As species and the maternal methylation efficiency, and the neuropsychological scores. We explored effect modification by sex, iron status, maternal nutrients status (serum manganese and selenium, and urinary zinc), and maternal vitamins intake (folate, and vitamins B_{12} and B_6).
Results: The geometric mean (95%CI) of ∑As (sum of DMA, MMA and iAs) was 7.78 (7.41, 8.17) μg/g creatinine. MMA concentrations were inversely associated with the scores for the general, verbal, quantitative, memory, executive function and working memory scales (i.e. β [CI95%] = −1.37 [-2.33, −0.41] for the general scale). An inverse association between %MMA and the memory scores was found. Children whose mothers had lower

* Corresponding author. Department of Preventive Medicine and Public Health of the University of the Basque Country, UPV/EHU, 48940, Leioa, Spain.
E-mail address: aitana.lertxundi@ehu.eus (A. Lertxundi).

https://doi.org/10.1016/j.envres.2021.112208
Received 7 May 2021; Received in revised form 13 September 2021; Accepted 9 October 2021
Available online 15 October 2021

manganese, zinc and ferritin concentrations obtained lower scores on several MSCA scales with decreasing As methylation efficiency.
Discussion: An inverse association was observed between MMA concentrations and children's neuropsychological development. Maternal levels of manganese, zinc and ferritin affected the association between As methylation efficiency and MSCA scores.

Abbreviations

AB	arsenobetaine
AIC	Akaike information criteria
As	arsenic
BBB	blood brain-barrier
BMI	body Mass Index
Cd	cadmium
CNS	central nervous system
DMA	dimethylarsinic acid
Fe	iron
FFQ	food frequency questionnaire
GAM	generalized additive models
GSH	glutathione
iAs	inorganic arsenic
ICPMS/MS	inductively coupled plasma tandem mass spectrometer
ID	iron deficiency
INMA	INfancia y Medio Ambiente (Childhood and Environment)
IQ	intelligence quotient
LCPUFA	long-chain polyunsaturated fatty acids
LOD	limit of detection
LRT	Likelihood Ratio test
MMA	monomethylarsonic acid
Mn	manganese
MSCA	McCarthy Scales of Children's abilities
NO2	Nitrogen Dioxide
oAs	organic arsenic
Pb	lead
PCA	principal component analysis
ROS	reactive oxygen species
SD	standard deviation
Se	selenium
TAs	total Arsenic
Zn	zinc
∑As	sum of DMA, MMA and iAs

1. Introduction

Arsenic (As) is a metalloid ubiquitously present in the environment. The inorganic form of this compound (iAs) is mainly found in soil and water. In regions with elevated iAs concentrations in soil and groundwater (i.e. some areas of Bangladesh, India, Mexico, Chile or USA), the main route of exposure to this element is through drinking water. However, the consumption of certain foods, especially rice but also other products such as molluscs or legumes, is the main route of exposure to this element in areas with low levels of As in water, as is the case in Spain (European Food Safety Authority, 2014; Soler-Blasco et al., 2021). The route of exposure to the organic forms (oAs), arsenobetaine (AB) and other complex forms of As, such as arsenolipids and arsenosugars, is mainly through seafood and fish consumption (Agence nationale de sécurité sanitaire de l'alimentation de l'environnement et du travail, 2011; Agència Catalana de Seguretat Alimentaria, 2020).

After ingestion, iAs is biotransformed through reduction and methylation processes, producing reduced (As^{V} to As^{III}) and methylated (monomethylarsonic acid [MMA] and dimethylarsinic acid [DMA]) forms (Challenger, 1945; Hayakawa et al., 2005). The relative concentrations of each metabolite excreted through the urinary system have been used as an estimation of individual methylation capacity, indicated by higher %DMA and lower %MMA and %iAs, especially in populations exposed to high levels of iAs through drinking water (Vahter, 1999). Several factors seem to affect methylation efficiency, such as the intake of some nutrients and elements. Particularly, evidence has also been found of the influence of nutrients that participate in one-carbon metabolism (OCM), such as vitamins B_6 and B_{12}, and folate, which are involved in the synthesis of S-adenosylmethionine (SAM), the main donor of the methyl group in iAs methylation (Kurzius-Spencer et al., 2017; Laine et al., 2018). Moreover, other essential elements appear to have an influence on As metabolism, such as manganese (Mn), selenium (Se) or zinc (Zn) (López-Carrillo et al., 2016; Rahman et al., 2019; Trasande et al., 2014; Valeri et al., 2016). Toxic elements, such as cadmium (Cd), also seem to be related to the efficiency of As metabolism, by binding to reduced glutathione, an antioxidant involved in the reduction process (Nordberg et al., 2005). Another factor that could affect the As metabolism is the pregnancy status. An increase in As methylation has been observed during pregnancy, denoted by a higher %DMA and lower %MMA (Gardner et al., 2011; Hopenhayn et al., 2003). This increase in As methylation efficiency appears more rapidly during the first trimester of pregnancy (Gardner et al., 2011). Regarding the most complex forms of As, AB is excreted without changes through the urine, but arsenosugars and arsenolipids seem to be metabolised, producing DMA (Molin et al., 2012; Taylor et al., 2017).

Inorganic As and its species are able to cross the placental barrier and be transferred from the mother to the fetus (Hall et al., 2007; Punshon et al., 2015). Exposure to this compound during prenatal development has been related to a decrease in birth size, an increase in respiratory symptoms and infections during childhood and an increase in the risk of neonatal mortality, particularly in areas where water is contaminated by arsenic (Sanchez et al., 2016; Zhong et al., 2019). Moreover, the developing brain seems to be vulnerable to this toxicant (Grandjean and Landrigan, 2006). The development of the central nervous system (CNS) is a process that begins very early in embryogenesis and continues until adolescence (Stiles and Jernigan, 2010). As and its compounds, which are able to cross the blood brain barrier, could produce neurotransmitter impairment, brain cell death and degeneration of CNS, among other toxic effects (Piao et al., 2015; Tolins et al., 2014). These prenatal changes seem to increase the susceptibility to develop negative effects in the neuropsychological development throughout childhood (Grandjean and Landrigan, 2006; Piao et al., 2015).

Some epidemiological studies have evaluated the relationship between prenatal exposure to As and neuropsychological development assessed during childhood, but the results are heterogeneous. A study carried out in Bangladesh found an inverse association between maternal urinary total As at 8 and 30 weeks of gestation (median = 81 μg/L and 84 μg/L, respectively) and the verbal and total scales of the Wechsler Preschool and Primary Scale of Intelligence at 5 years of age.

Nevertheless, this relation was found only in girls (Hamadani et al., 2011). A Spanish study observed an inverse association between placental total As levels (median= <0.23 ng/g) and the scores for the global executive and verbal scales of the McCarthy Scales of Children's abilities (MSCA) at 4 years of age (Freire et al., 2018). Conversely, another Spanish study did not observe any significant association between maternal total As, measured in urine in the first and third trimesters of pregnancy (median = 27.10 and 29.80 μg/L, respectively), and the MSCA at 4 years of age (Forns et al., 2014). Similarly, a Nepalese cohort study found no evidence of association between cord blood total As (median = 1.46 μg/L) and child neurodevelopment at 6, 24 and 36 months of age (Parajuli et al., 2014, 2015a, 2015b). This heterogeneity in the results of the association between prenatal As and neurodevelopment could be related to the biomarker used in most of the studies, total As (sum of organic and inorganic forms) or ∑As (sum of DMA, MMA and iAs). Nonetheless, there is evidence that the intermediate As compounds could have higher reactivity and toxicity (Mass et al., 2001; Styblo et al., 2000). Therefore, more longitudinal birth cohorts are needed in order to assess the relationship between the different As species and children's neuropsychological development.

The aim of this study is to explore the relationship between prenatal concentrations of total As (TAs) as well as the different urinary As species (arsenobetaine [AB], DMA, MMA and iAs) and the methylation efficiency in the first trimester of pregnancy and children's neuropsychological development assessed at 4–5 years of age in a Spanish birth cohort. We also studied the effect modification produced by sex, the maternal levels of certain nutrients and elements (serum Mn, Se and ferritin, and urinary Zn), as well as the intake of vitamins (folate and vitamins B_6 and B_{12}) during pregnancy.

2. Material and methods

2.1. Study population

The participants in the study were mother–child pairs from the INMA (Childhood and Environment) Project, a multicentre birth cohort study that aims to investigate the effect of environmental exposures and diet during pregnancy and childhood on fetal and child development in different geographical areas of Spain (http://www.proyectoinma.org). Subjects were participants in the Gipuzkoa (northeast of Spain) and Valencia (east of Spain) areas. The study protocol has been reported elsewhere (Guxens et al., 2012). Briefly, 1493 pregnant women were recruited during their first antenatal visit (2003–2008, Gipuzkoa: 638, Valencia: 855). Fig. 1 shows the process of selecting the participants in the present study. The final study population was made up of 807 mother–child pairs in whom urinary arsenic species concentrations in the first trimester of pregnancy and neuropsychological assessment at age 4–5 years were both available.

The study protocol was approved by the Ethics Committee of the University Hospital La Fe, the Ethics Committee of the Public Health Research Centre of Valencia (Valencia) and the Ethics Committee of Donostia Hospital (Gipuzkoa). Informed consent regarding the prenatal period was signed by the mother and in each phase of the postnatal period further consent was signed by one of the parents or a legal representative.

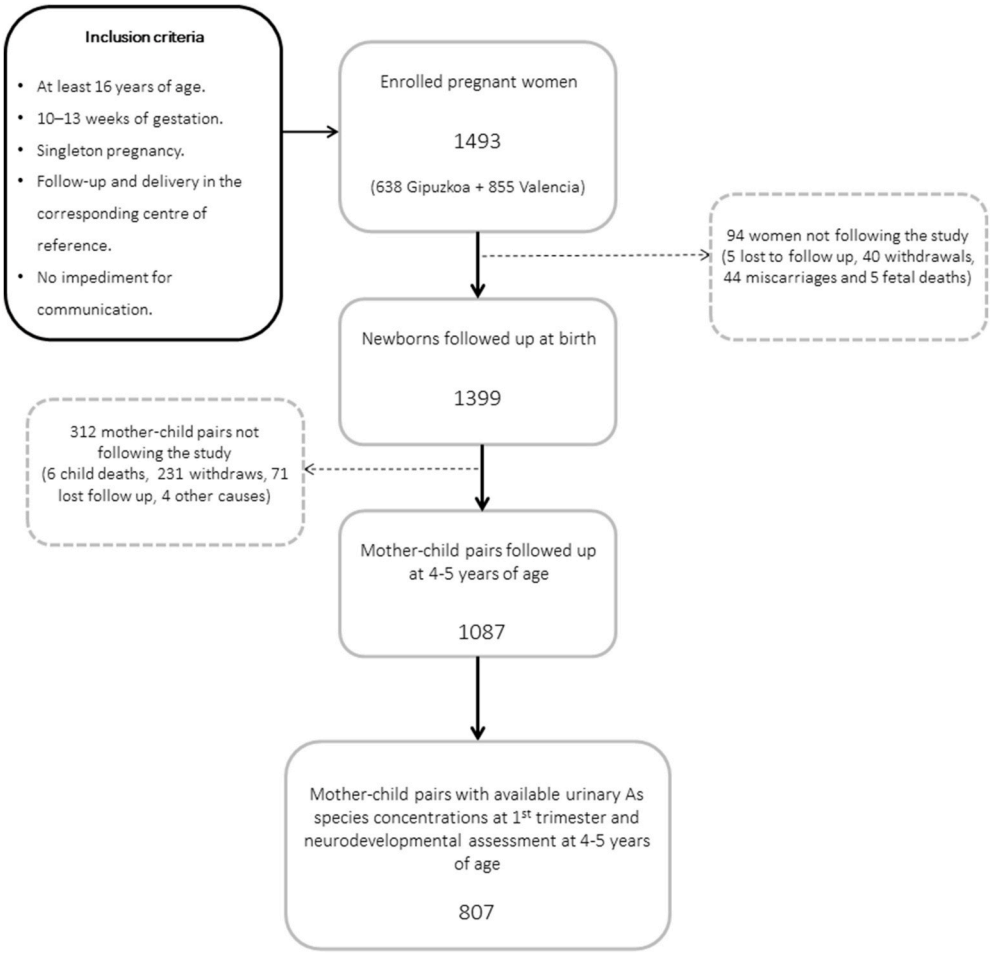


Fig. 1. Flow chart describing the process of selecting participants in the INMA Project (Valencia and Gipuzkoa, Spain, 2003–2008) to be included in the present analysis.

2.2. Study variables and sources of information

2.2.1. Exposure variable: urinary arsenic speciation analysis

Concentrations of TAs and its metabolites were determined in spot urine samples collected in the first trimester of pregnancy (mean [standard deviation, SD] = 13.1 [1.5] weeks of gestation). Urine samples were kept frozen at −80 °C until their analysis. The TAs concentrations were determined with an inductively coupled plasma tandem mass spectrometer (ICPMS/MS, 8800, Agilent Technologies, Waldbronn, Germany). For the speciation analysis, high performance liquid chromatography (1200, Agilent Technologies) coupled to ICPMS/MS (8800, Agilent Technologies) was employed. More details about the laboratory technique have been described in Soler-Blasco et al. (2021) and in Appendix S1 (A.1.1 Description of urinary arsenic speciation analysis). Limits of detection (LOD) of AB, DMA, MMA and iAs were 0.02, 0.03, 0.03 and 0.03 μg/L, respectively. Where samples were below LOD, ½ LOD was assigned for the statistical analysis (2.6% of samples for MMA levels and 2.2% of samples for iAs levels).

2.2.2. Outcome variable: neuropsychological assessment

Neuropsychological development of the children was assessed at around 5 years of age (mean [SD] = 5.2 [0.7]) using the McCarthy Scales of Children's Abilities (MSCA) adapted to the Spanish population (McCarthy, 2009). The original MSCA contains eighteen subtests grouped into a general scale and five global sub-area scales. The verbal scale refers to cognitive tasks related to the processing of verbal information; the quantitative scale assesses numerical abilities; the perceptive-performance scale refers to cognitive tasks related to perceptual information processing, including manual performance; the memory scale considers short-term retention of information (verbal, visual or numerical); and the motor scale refers to fine (e.g. drawing) and gross (e.g. balance or accuracy) abilities. The sum of the first three scales provides the general cognitive scale. In addition, some items were used to construct a new summary measure to assess those cognitive tasks associated with executive function. The new sub-areas of MSCA used in this study were gross motor and fine motor skills, executive function and working memory (Julvez et al., 2011). Higher scores on the general scale and sub-scales indicate better cognitive development.

Testing was conducted in the research centres of each area of study by six psychologists using a strict protocol to avoid inter-observer variability. This protocol included inter-observer training and sets of quality control (inter-observer reliability-tests). The inter-observer variability assessed by Pearson correlations was lower than 5%. The raw scores were standardized for the child's age at test administration and for psychologist. In order to homogenize scales, standardized residuals were then typified by having a mean of 100 and a standard deviation of 15 points.

2.2.3. Covariates and potential confounders

The women filled in two questionnaires during their pregnancy, in the first and the third trimesters of gestation (mean [SD] = 13.1 [1.5] and 32.4 [2.1] weeks of gestation, respectively). Sociodemographic, environmental and lifestyle information was collected through questionnaires filled in during pregnancy and later at 12–14 months of age and at 4–5 years. More information about the covariates used in this study can be found in Supplemental material Appendix S1 (A.1.2 Description of covariables and potential confounders). Information on the child's sex and gestational age at birth was obtained from clinical records.

Dietary intake was assessed in the first trimester of pregnancy using a validated semi-quantitative food frequency questionnaire (FFQ) (Vioque et al., 2013). We calculated the consumption expressed in grams per day for those foods previously related to the As species in our population (Soler-Blasco et al., 2021) such as rice, other cereals, meat, seafood and shellfish, vegetables and legumes. Energy-adjusted intakes were computed using the residual method (Willet, 2013). Additionally, dietary folate and vitamins B_{12} and B_6 intakes were also estimated using the food composition tables of the US Department of Agriculture (U.S. Department of Agriculture: Agricultural Research Service USDA, 2007) and with Spanish sources (Palma et al., 2008). The intake of supplements was added to the calculation of total daily nutrient intake (Vioque et al., 2013).

Serum manganese (Mn) and selenium (Se), urinary cadmium (Cd) and zinc (Zn), and plasma ferritin concentrations were determined from the first trimester of pregnancy. More information about the methodology has been reported in detail elsewhere (Arija et al., 2019; Lozano et al., 2020; Soler-Blasco et al., 2020). Creatinine concentrations were measured in the same urine samples in the first trimester of pregnancy by the DRI® Creatinine-Detected® Test using AV680 from Beckman Coulter.

Nitrogen dioxide (NO_2) concentrations in the first trimester of pregnancy were measured to estimate personal exposure to traffic-related air pollution. Ambient concentrations were measured through passive samplers (Radiello, Fundazione Salvatore Maugeri, Padua, Italy) distributed according to geographic criteria. The samplers remained exposed throughout various 7-day sampling periods. More information about the methodology can be found in Iniguez et al., 2009.

2.3. Statistical analysis

We calculated the geometric mean (GM) and 95% confidence intervals (95%CI) of the urinary creatinine adjusted (μg/g creatinine) TAs, AB, DMA, MMA, iAs and ∑As concentrations (as the sum of iAs, DMA and MMA). GM and 95%CI of the measured TAs and ∑s were calculated according to sociodemographic, environmental and dietary characteristics of the study population. Descriptive analyses of the rest of the As species according to the characteristics of the study population can be seen in Soler-Blasco et al. (2021). For further analysis, we applied the $\log_2$ and probit-transform functions on values of the urinary As species concentrations and the percentage of the individual metabolites, respectively, in order to correct their skewed distribution.

High consumption of seafood can lead to an inappropriate estimation of iAs exposure through the sum of the As species (DMA + MMA + iAs), because the organic arsenic (oAs) from seafood contributes to DMA levels and TAs (Navas-Acien et al., 2011). To minimize this, we obtained new calibrated iAs, MMA and DMA concentration variables using the mathematical method proposed by Jones et al. (2016). More information about this method can be found in Soler-Blasco et al. (2021) and in Supplemental material Appendix S1 (A.1.3 Description of the arsenic species calibration methodology).

Arsenic methylation efficiency was determined through two approaches. The first consists in calculating the percentage of the individual calibrated iAs, MMA and DMA over the sum of those species (∑As). The second involves a principal component analysis (PCA) of the three calibrated, untransformed and un-rotated percentages corrected for maternal creatinine. The PCA was performed to avoid the high correlation between the percentages of the three metabolites. The results obtained through the PCA were two principal components (PC1 and PC2) that explain 100% of the original variance. Therefore, these two PC were used as As methylation phenotypes.

In order to assess the relation between prenatal exposure to the different As species, including the maternal methylation capacity, and the neuropsychological test scores, multivariable linear regression models for each of the ten scales were built separately with the following procedure. In the first step, (I) core models were built for each scale with parental and child sociodemographic variables associated with a p-value <0.2 in the bivariate analysis. Following a backward elimination procedure, all the covariates associated with each neuropsychological test scale at a p-value level <0.1 in the likelihood ratio test were retained in the model. The area of study (Valencia or Gipuzkoa) and maternal urinary creatinine concentrations were included in all core models regardless of their statistical significance. In the second step (II), each As

variable (uncalibrated, unadjusted for urinary creatinine and log2 transformed TAs, ∑As, AB, DMA, MMA and iAs concentrations, calibrated and probit-transformed DMA, MMA and iAs percentages, and PC1 and PC2) were included one by one in these models as exposure variables. Additional potential confounders were also included if they changed the effect of each exposure variable in a significant way compared to the same potential confounder, but randomized (that is, the same variable randomly reordered to simulate independence from each exposure variable and the response variable), with a 5% significance level (Lee, 2014). We tested all the covariates associated with the different As species as possible confounders in a previous study (Soler-Blasco et al., 2021).

Effect modification analysis was performed by creating an interaction term between the exposure variable and the potential modifier: child's sex, maternal nutrients categorized by the median: Mn (<and ≥1.44 μg/L), Se (<and ≥79.8 μg/L) and Zn (<and ≥363.7 μg/L), intake of vitamins B_6 (<and ≥2.3 mg/day) and B_{12} (<and ≥9.4 mg/day), ferritin (categorized as iron deficiency [ID] as < and ≥15 μg/L), and intake of folate (categorized by the intake recommendation during pregnancy, < and ≥600 μg/day]. The models with and without an interaction term were compared with the Likelihood Ratio test (LRT) and the effect modification was considered statistically significant if the p-value <0.05.

Finally, Generalized Additive Models (GAM) were fitted to evaluate non-linear patterns on the association between As species and scores on the MSCA sub-scales. Natural cubic splines with one or two internal knots were compared through Akaike Information Criteria (AIC). The non-linear model and the linear model, both with the lowest AIC, were then compared by graphical examination and the LRT.

Several sensitivity analyses were performed to evaluate the robustness of the multivariable models by eliminating certain population subgroups from the sample data set: preterm birth (<week 37 of gestation, n = 31), low birth weight (<2500 g, n = 39), or those in whom the neuropsychological test quality was uncertain, namely tests on children who presented special conditions while completing them (e.g. behavioural problems, sleepiness or being feverish) (n = 27). Furthermore, the multivariable models were built using the non-calibrated As metabolite percentages as exposure variables instead of the calibrated ones, and also including the maternal fish consumption variable in the models. Finally, two additional analyses were performed in order to evaluate the possible confounding effect of maternal smoking habit: 1) multivariate models were adjusted for maternal tobacco consumption during the first trimester of pregnancy, and 2) the maternal smoking habit was evaluated as a potential modifier by interaction terms.

Validation of the linear regression models was addressed by analysing the residuals: we visually inspected model residuals for normality and homoscedasticity. No influential data was identified by Cook's distance. Variance inflation factors (VIFs) were used as a check for collinearity among variables in the final models, all VIFs being <2.5.

Statistical analyses were conducted using the R statistical package version 4.0.3 (R Core Team, 2020).

3. Results

3.1. Description of TAs and its metabolite concentrations and as methylation efficiency

Differences between included and excluded subjects are shown in Table S1. The participating mothers were slightly older and had a higher level of education and social class than the non-participants. The GM (95%CI) of maternal urinary TAs, AB and ∑As were 35.75 (33.01, 38.73), 20.72 (18.66, 23.01) and 7.78 (7.41, 8.17) μg/g creatinine, respectively. The concentrations of each metabolite were 6.85 (6.51, 7.21) μg/g creatinine for DMA, 0.34 (0.32, 0.36) μg/g creatinine for MMA and 0.33 (0.31, 0.35) μg/g creatinine for iAs. The medians (95% CI) of the calibrated %DMA, %MMA and %iAs were 84.47% (84.00, 85.04), 7.07% (6.67, 7.40) and 7.55% (7.29, 7.93), respectively (Table S2).

The variability of the three calibrated percentages of metabolites can be summarized in two principal components. Principal component 1 (PC1) explained 89% of the variance and was characterized by higher %DMA and lower %iAs and %MMA. Principal component 2 (PC2) explained the remaining 11% of the variance and was characterized by higher %iAs and lower %MMA.

3.2. Association between TAs, its metabolites and as methylation efficiency and MSCA scores

Maternal urinary MMA concentrations were linear, inverse and significantly associated with the general scale (β [95%CI]: 1.37 [−2.33, −0.41], p-value = 0.01), verbal scale (β [95%CI]: 1.18 [−2.13, −0.23], p-value = 0.02), quantitative scale (β [95%CI]: 1.23 [−2.20, −0.27], p-value = 0.01), memory scale (β [95%CI]: 1.19 [−2.17, −0.20], p-value = 0.02), executive function scale (β [95%CI]: 0.98 [−2.00, 0.04], p-value = 0.06) and working memory scale (β [95% CI]: 0.96 [−1.90, −0.02], p-value = 0.04) (Fig. 2). No associations were found between the other As metabolites analysed and the MSCA scales in the multivariate models, except for TAs and AB concentrations, which were positively associated with the verbal scale (β [95%CI]: 0.65 [0.03, 1.27], p-value = 0.04 and 0.59 [0.11, 1.07], p-value = 0.02, respectively) (Fig. 2). Finally, in order to investigate the positive association between maternal urinary TAs and AB and the scores of the verbal scale, we ran the main models in a sub-sample of pregnant women with the least seafood consumption (first quartile of seafood consumption = 0–25.22 g per week, n = 201). The positive coefficients were maintained but the p-value of the associations did not reach statistical significance (see Table S5).

Regarding As methylation efficiency, the model showed an inverse association between calibrated %MMA and the memory scale scores (β [95%CI]: 3.33 [−6.72, 0.05], p = 0.05), this relation being U-shaped (p-value (LRT) for the spline-based model <0.01). Furthermore, a positive relationship was observed between calibrated %iAs and the scores for the gross motor scale (β [95%CI]: 2.32 [−0.03, 4.66], p = 0.05) (Fig. 3). Finally, PC1 and PC2, used as methylation phenotypes, were not related to any MSCA scale (Fig. 3).

3.3. Effect modification analysis

Fig. 4 and Table S3 show the influence of child's sex and maternal nutrients (serum Mn, Se and ferritin, urinary Zn, and estimated vitamins B_6 and B_{12} and folate intake) on the association between methylation efficiency and the scores for the ten MSCA scales. Regarding child's sex, we observed that boys whose mothers had higher %DMA obtained lower scores on the executive function scale (β [95%CI] = −4.05 [−7.78, −0.32], interaction p-value = 0.085), compared to girls (β [95%CI] = 0.74 [−3.28, 4.76]). Also, boys whose mothers had higher %iAs obtained lower scores on the fine motor scale (β [95%CI] = −2.059 [−5.257, −1.140], interaction p-value = 0.084), compared to girls (β [95%CI] = 1.99 [−1.32, 5.29]). Nevertheless, neither of the p-values of the interaction term was significant.

We observed statistically significant interactions between maternal serum Mn and %DMA, %MMA and %iAs, but with different patterns. Specifically, children whose mothers had Mn concentrations below the median (<1.44 μg/L) obtained lower scores on the perceptual performance scale with increasing %MMA (β [95%CI] = −3.82 [−8.57, −0.93], interaction p-value = 0.03) and on the fine motor scale with increasing %iAs (β [95%CI] = −2.47 [−5.62, 0.67], interaction p-value = 0.02). Moreover, children whose mothers had Mn concentrations above the median obtained lower scores on the motor scale with increasing %DMA (β [95%CI] = −4.45 [−8.33, −0.57], interaction p-value = 0.05).

Other statistically significant interactions observed were between

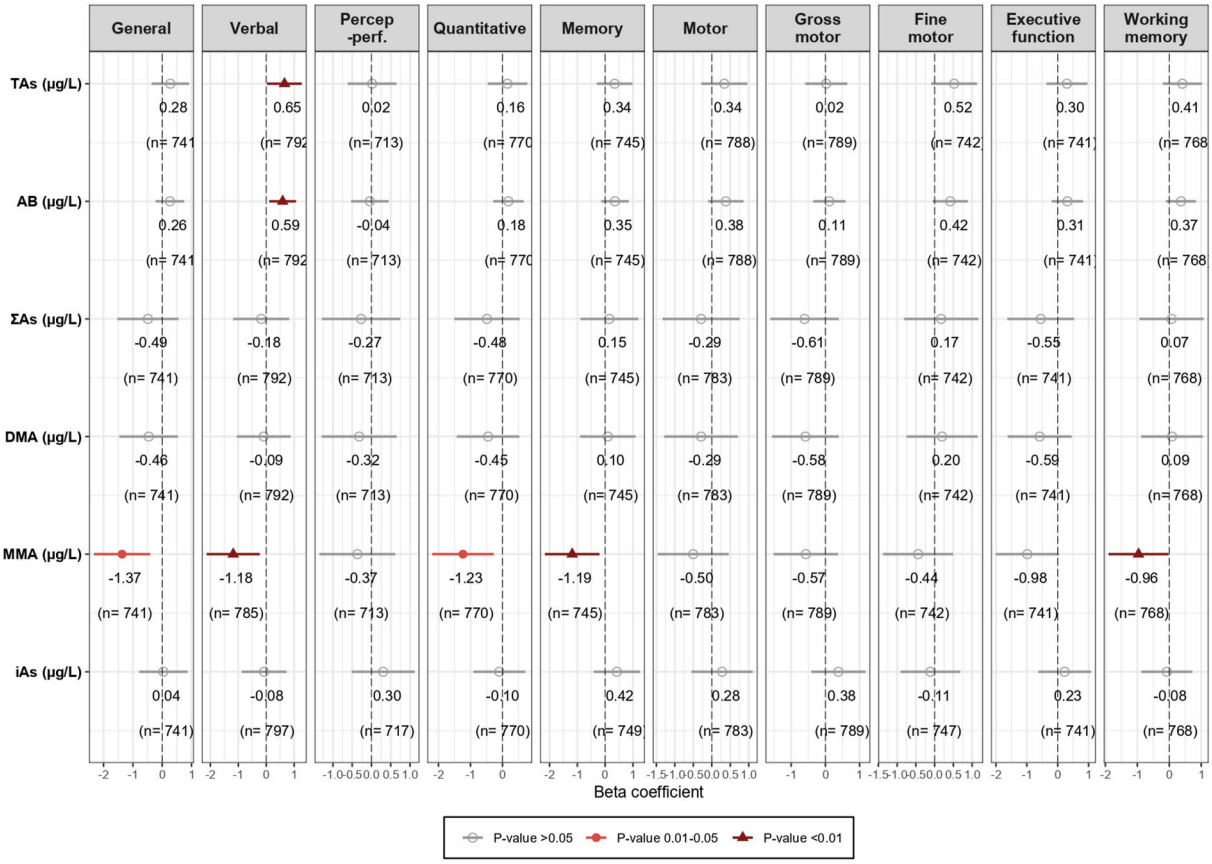


Fig. 2. Association between prenatal As and its metabolite concentrations and children's neuropsychological development assessed by the McCarthy test scores at 4–5 years of age. INMA Project (Valencia and Gipuzkoa. Spain. 2003–2008). Note: Percept-Perform, perceptive-performance; µg/L, micrograms per litre; TAs, Total As; ΣAs, sum of DMA, MMA and iAs; DMA, dimethylarsinic acid; MMA, monomethylarsonic acid; iAs, inorganic As; %DMA, percentage of dimethylarsinic acid; %MMA, percentage of monomethyl arsonic acid; %iAs, percentage of inorganic As; n = total sample used in each model. As and its metabolite concentrations were log2-transformed. All models were adjusted for creatinine. Additionally, each model was adjusted for different confounders and covariates (See Tables S4.1, S4.2 and, S4.3).

maternal urinary Zn and %MMA. Thus, children whose mothers had urinary Zn concentrations below the median (<363.8 µg/L) obtained lower scores on the motor, fine motor and gross motor scales with increasing %MMA (β [95%CI] = −3.44 [−7.64, 0.75], interaction p-value <0.01, β [95%CI] = −2.32 [−6.38, 1.74], interaction p-value = 0.03 and β [95%CI] = −4.06 [−8.14, 0.02], interaction p-value = 0.01, respectively). Additionally, the interaction between maternal ferritin and %iAs was also statistically significant for three of the scales. Children whose mothers presented ID (serum ferritin <15 µg/L) obtained worse scores for the general, verbal and memory scales with increasing %iAs (β [95%CI] = −4.27 [−10.83, 2.29], p interaction = 0.04, β [95% CI] = −6.58 [−13.13, −0.03], p interaction = 0.01 and β [95%CI] = −6.33 [−12.94, 0.26], p interaction = <0.01, respectively).

3.4. Sensitivity analysis

The exclusion of preterm births (n = 31), low birth weight (n = 39) or those in whom the quality test was uncertain (n = 27) did not affect the estimates meaningfully (Fig. S1 and Fig. S2).

The same analysis was performed using the non-calibrated As metabolite percentages, a slightly lower β coefficient being observed, together with a statistically significant p-value in the association between %MMA and on the working memory scale (Fig. S3). When the maternal fish intake variable was included in the models, the results were virtually the same. Finally, when we included the variable of the maternal smoking habit in the main models of the general, verbal, quantitative, memory and executive function scales, the $\log_2$ MMA estimates were not affected (Table S6). In the same way, we did not observe any statistically significant interactions between maternal smoking habit and log2 MMA concentrations (Fig. S4).

4. Discussion

The present study, carried out on the INMA Spanish birth cohort, found an inverse association between maternal urinary MMA concentrations and general, verbal, quantitative, memory, executive function and working memory development at 4–5 years of age. Additionally, %MMA was inversely associated with the memory scale scores. The association between As methylation and children's neuropsychological development seems to be influenced by the maternal concentrations of some nutrients and elements; thus, children whose mothers had lower levels of Mn, Zn and ferritin during pregnancy obtained worse scores with decreasing As methylation efficiency. As far as we know, this is the largest epidemiological study to date evaluating the association between prenatal As metabolites and neuropsychological development in childhood in an area with low-level exposure to As (World Health Organization, 2017).

Some birth cohort studies have evaluated the relation between prenatal As exposure and neuropsychological development in childhood (Table 1). In Bangladesh, an inverse association was found between urinary ∑As concentrations in the first trimester of pregnancy (median [p10, p90] = 81 [24,380] µg/L) and the verbal intelligence quotient scale at 5 years of age, assessed by the Wechsler Preschool and Primary Scale of Intelligence. Nevertheless, this inverse association was only

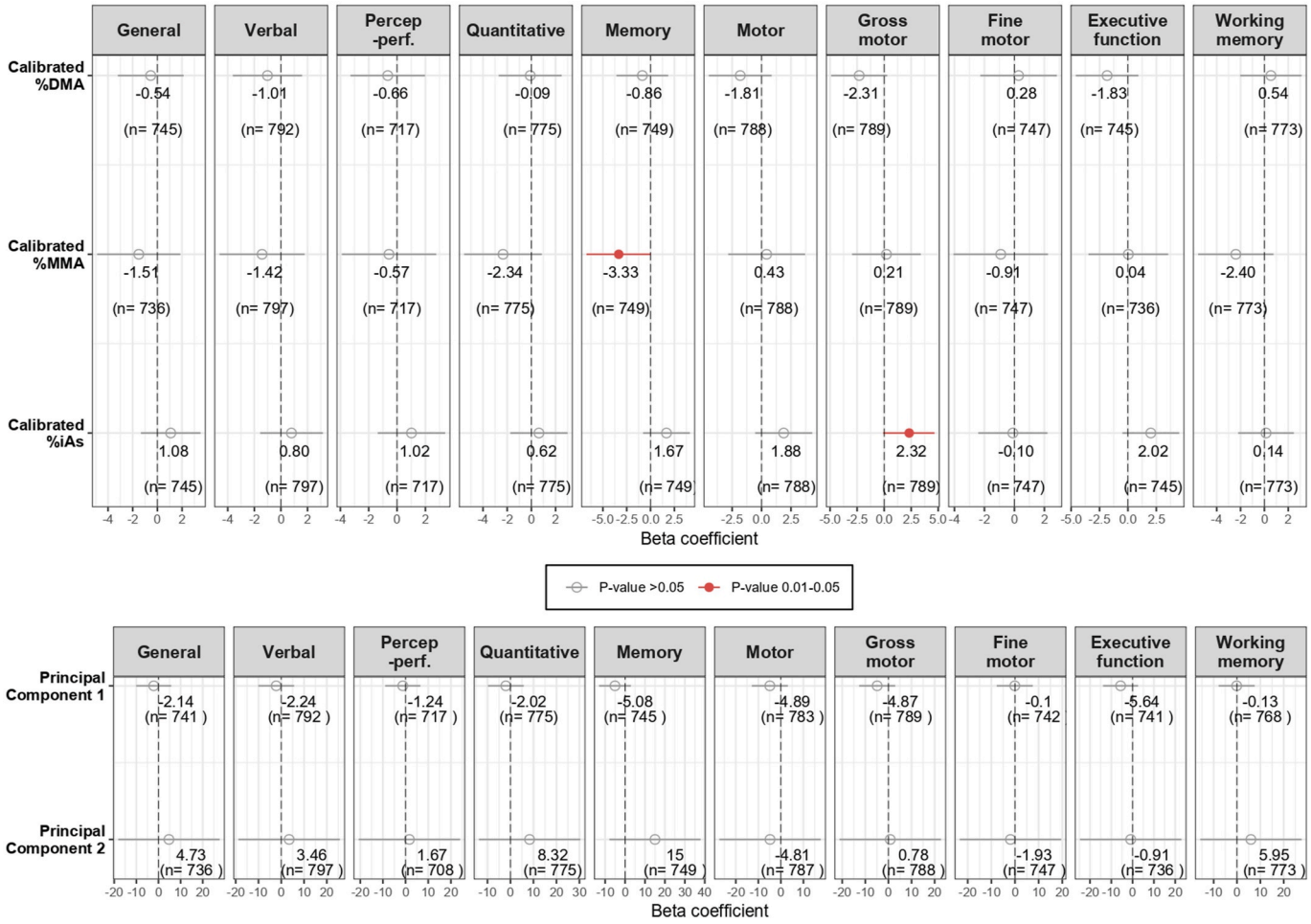


Fig. 3. Association between As methylation efficiency (measured by calibrated[1] As metabolite percentages and principal components 1 and 2) and children's neuropsychological development assessed by the McCarthy test scores at 4–5 years of age. INMA Project (Valencia and Gipuzkoa. Spain. 2003–2008). Note: Percept-Perform, perceptive-performance; %DMA, percentage of dimethylarsinic acid; % MMA, percentage of monomethyl arsonic acid; %iAs, percentage of inorganic As; PC: principal component; n = total sample used in each model. [1]Calibrated percentages were calculated with As metabolite concentrations corrected for arsenobetaine and creatinine concentrations. The percentages of each metabolite were calculated: levels of calibrated metabolite/(calibrated DMA + calibrated MMA + calibrated unmethylated iAs). Calibrated percentages were probit-transformed. Additionally, each model was adjusted for different confounders and covariates (See Tables S4.1, S4.2 and, S4.3).

observed for girls (Hamadani et al., 2011). In the same cohort, prenatal ∑As was inversely associated with verbal comprehension, processing reasoning, processing speed and the full development score at 10 years of age (Vahter et al., 2020). In another birth cohort study carried out in Nepal, the association between prenatal As exposure measured in cord blood (median [p25, p75] = 1.46 [0.97, 1.74] μg/L) and child neurodevelopment at birth and at 6 and 24 months of age was evaluated. The researchers only found an inverse association between cord blood As and the state regulation cluster scores of the Brazelton neonatal behavioural assessment; no association with child neurodevelopment was observed at 6 and 24 months of age (Parajuli et al., 2013, 2014, 2015a). In the Ma'anshan-Anhui Birth Cohort (China), As cord blood concentrations (serum) (median [p25, p75] = 1.89 [1.27, 2.89] μg/L) were associated with a higher risk of developmental delay in the social domain among 6-month-old children, assessed through the Ages and Stages Questionnaire. Nevertheless, this association was only significant in girls (Liang et al., 2020). Finally, in a previous study in the INMA cohort an inverse association was observed between placenta As concentrations (median <0.23 ng/g) and executive function and verbal executive function, evaluated through the MSCA at 5 years of age (Freire et al., 2018) (see Table 2).

Thus, the epidemiology literature on the relationship between prenatal As exposure and neuropsychological development in childhood is still too scarce to draw any definitive conclusion. Additionally, the methodology used in the different studies is too heterogeneous, which hampers comparability between them. Furthermore, the biomarker of exposure used in these previous studies was total As (sum of organic and inorganic forms) or ∑As (sum of DMA, MMA and iAs), and none of them evaluated the different species individually. This fact may have added a certain degree of imprecision in the exposure assessment that could partly explain the inconsistencies in the results. Arsenic concentrations were substantially different across the studies; for example, the median urinary ∑As concentrations in the Bangladeshi cohort of pregnant women were around 80 μg/L, which are much higher concentrations than in the present study (7.4 μg/L). These differences in urinary ∑As concentrations could imply a differential relative contribution of each species in the total concentrations. For example, in the Bangladeshi birth cohort, the major contributor in ∑As may be iAs, due to the fact that the general population in Bangladesh is highly exposed to iAs through drinking water (Shahid et al., 2020). In contrast, in our population, iAs exposure from water is low (around 1 μg/L) (Ministerio de Sanidad Servicios Sociales e Igualdad, 2019), and the main contributor to prenatal iAs and its metabolite concentrations is the consumption of certain foods, such as rice or molluscs (Soler-Blasco et al., 2021). Moreover, our population was highly exposed to non-toxic organic arsenicals from high fish consumption.

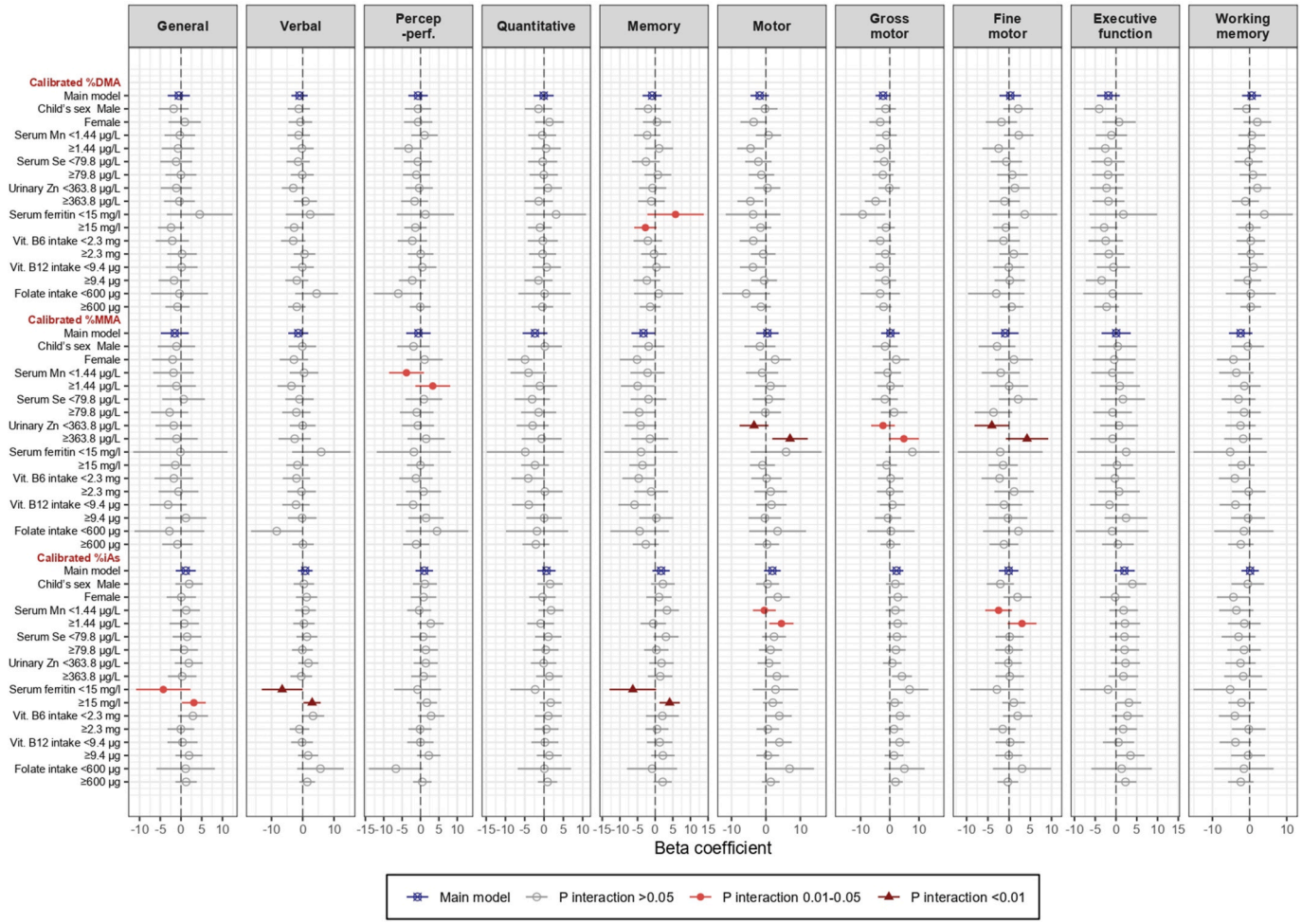


Fig. 4. Effect modification of some child and maternal factors on the association between the calibrated As metabolite percentages and the child neuropsychological development assessed by the McCarthy test scores at 4–5 years of age. INMA Project (Valencia and Gipuzkoa, Spain. 2003–2008). Note: Percept-Perform: perceptive-performance; %DMA, percentage of dimethylarsinic acid; % MMA, percentage of monomethylarsonic acid; %iAs, percentage of inorganic As; Mn, manganese; Se, selenium; Zn, zinc; Vit, vitamin. [a] As metabolite concentrations corrected for arsenobetaine and creatinine concentrations. All models were adjusted for creatinine. Additionally, each model was adjusted for different confounders and covariates (See Tables S4.1, S4.2 and, S4.3). The percentages of each metabolite were calculated: levels of the calibrated metabolite/(calibrated DMA + calibrated MMA + calibrated iAs)*100.

In our study, maternal urinary MMA concentrations were associated with a decrease in the scores of several MSCA scales. We have not found any other studies that have analysed the relationship between prenatal exposure to different arsenic metabolites and neuropsychological development during childhood. Only a few cross-sectional studies have analysed the association between postnatal urinary metabolites and children's cognitive function. In one Mexican study (n = 602), the authors found a significant association between urinary MMA concentrations (mean = 7.7 µg/L) and problem-solving, vocabulary, memory and attention tests in schoolchildren, evaluated by several tests (Letter Sequencing, Visual Search, the Peabody Picture Vocabulary Test, the Weschsler Intelligence Scale for Children Revised Mexican Version Digit Span Subscale, among others) (Rosado et al., 2007). In a case-control study carried out in Taiwan (n of cases = 63, n of controls = 35), the MMA and the iAs concentrations in the highest tertile (>0.0028 and > 0.49 µg/L, respectively) increased the risk of neurodevelopmental delay in preschool children, evaluated through multiple neurodevelopmental tests (Peabody Developmental Motor Scales, Gross Motor Function Measure, Chinese Wechsler Intelligence Scale for Children, and Bayley III Scales of Infant and Toddler Development, among others) (Hsieh et al., 2014). The results derived from these two studies with postnatal As exposure seem to be in agreement with those observed in our study with prenatal As. Nevertheless, comparison of the studies must be performed with caution.

Although the mechanism of As neurotoxicity is still not fully understood, some experimental studies on animals have proposed oxidative stress as a possible mechanism (Chandravanshi et al., 2018; Luo and Shu, 2015); As has been related to an increase in reactive oxygen species (ROS) concentrations and its accumulation in certain areas of the central nervous system, particularly in the frontal cortex region (Luo and Shu, 2015; Mishra and Flora, 2008). This accumulation causes lipid peroxidation, which leads to DNA damage and, consequently, brain cell death and degeneration of the CNS (Chandravanshi et al., 2018; Luo and Shu, 2015). The prefrontal cortex region is related to memory, perception, new learning and other cognitive processes (Siddiqui et al., 2008). Another As neurotoxicity mechanism that has been proposed is a neurotransmission impairment, specifically in the metabolism of acetylcholine. The cholinergic alteration produced seems to generate learning and memory impairment (Chandravanshi et al., 2014). The methylated forms, DMA and MMA, seem to be accumulated in the brain. Experimental studies have observed higher levels of MMA in the hippocampus, related to verbal learning and memory, and the thalamus, related to some motor tasks, working memory, attention control, learning and memory processing (Georgescu et al., 2020; Li et al., 2020; Sánchez-Peña et al., 2010; Tyler and Allan, 2014).

Although As metabolism is considered a detoxification mechanism, a

Table 1
Sociodemographic and environmental characteristics of study participants, and urinary maternal TAs and ∑As at first trimester.

Variables at pregnancy	Included Population (N = 807)	TAs (μg/g creatinine)	P-value[a]	∑As (μg/g creatinine)	P-value[a]
	N (%)				
Area of study					
Gipuzkoa	340 (42)	41.31 (36.39, 46.89)	<0.01	7.44 (6.91, 8.01)	0.13
Valencia	467 (58)	32.20 (29.08, 35.66)		8.04 (7.53, 8.58)	
Maternal age (years)					
<25	36 (4)	17.97 (12.41, 26.02)	<0.01	6.19 (4.94, 7.76)	0.17
25-29	263 (33)	33.91 (29.43, 39.07)		7.63 (6.99, 8.34)	
30-34	361 (45)	39.11 (34.91, 43.82)		7.98 (7.42, 8.58)	
≥35	147 (18)	37.31 (30.61, 45.47)		8.00 (7.15, 8.96)	
Maternal country of birth					
Spain	764 (95)	36.47 (33.62, 39.56)	0.01	7.73 (7.35, 8.13)	0.22
Others	43 (5)	25.11 (17.08, 36.91)		8.73 (6.99, 10.90)	
BMI before pregnancy (kg/m^2)					
≤25 (low and healthy weight)	610 (76)	37.89 (34.52, 41.58)	0.08	7.97 (7.54, 8.43)	0.28
25- <30 (overweight)	137 (17)	30.63 (25.64, 36.59)		7.22 (6.42, 8.13)	
≥30 (obesity)	60 (7)	28.26 (20.93, 38.15)		7.19 (5.87, 8.81)	
Parity					
0	446 (55)	37.08 (33.35, 41.24)	0.25	7.77 (7.26, 8.31)	0.76
≥1	361 (45)	34.18 (30.28, 38.58)		7.80 (7.26, 8.37)	
Parental social class					
I + II (high)	284 (35)	39.12 (34.15, 44.81)	0.17	8.10 (7.46, 8.80)	0.09
III	214 (27)	32.54 (28.27, 37.45)		7.09 (6.42, 7.83)	
IV + V (low)	309 (38)	35.13 (30.67, 40.24)		7.99 (7.40, 8.63)	
Maternal educational level					
Up to primary	168 (21)	30.21 (25.33, 36.03)	0.07	7.27 (6.52, 8.09)	0.30
Secondary	325 (40)	36.43 (32.20, 41.21)		8.09 (7.48, 8.75)	
University	313 (39)	38.28 (33.61, 43.60)		7.75 (7.17, 8.38)	
Paternal educational level					
Up to primary	269 (34)	31.70 (27.66, 36.34)	0.08	7.37 (6.80, 7.99)	0.46
Secondary	357 (44)	7.69 (33.40, 42.52)		7.90 (7.34, 8.50)	
University	175 (22)	38.06 (31.96, 45.33)		8.24 (7.35, 9.23)	
Maternal working status					
Non-worker	166 (21)	32.24 (26.73, 38.89)	0.11	7.48 (6.78, 8.26)	0.62
Worker	641 (79)	36.72 (33.63, 40.10)		7.86 (7.43, 8.32)	
Proximity of the residence to agricultural area					
No	469 (59)	36.68 (32.90, 40.89)	0.44	7.49 (7.02, 7.98)	0.06
Yes	329 (41)	34.27 (30.44, 38.59)		8.19 (7.57, 8.85)	
Maternal tobacco consumption[b]					
No	653 (82)	36.46 (33.36, 39.84)	0.15	7.87 (7.44 8.31)	0.26
Yes	143 (18)	31.44 (26.05, 37.95)		7.29 (6.50 8.17)	
Maternal alcohol consumption[b]					
No	691 (86)	35.21 (32.30, 38.39)	0.35	7.68 (7.28 8.10)	0.05
Yes	111 (14)	39.59 (31.87, 49.18)		8.59 (7.58 9.74)	
Rice consumption	48.8 (30.5)				
<1 serving per week	401 (50)	36.24 (32.25, 40.74)	0.68	7.14 (6.65 7.66)	<0.01
≥1 serving per week	401 (50)	35.34 (31.65, 39.46)		8.51 (7.95 9.11)	
Fish consumption	80.0 (35.1)				
<1 serving per week	180 (22)	23.23 (19.88, 27.14)	<0.01	6.79 (6.15 7.49)	<0.01
1 serving per week	347 (43)	37.26 (32.98, 42.09)		7.54 (7.02,8.09)	
>1 serving per week	275 (35)	45.15 (39.43, 51.71)		8.91 (8.14,9.74)	
Vitamin B_6 intake					
<2.3 mg/day	401 (50)	31.42 (26.41, 37.38)	0.09	7.05 (6.33, 7.86)	0.02
≥2.3 mg/day	401 (50)	37.20 (33.99, 40.71)		8.03 (7.60, 8.49)	
Vitamin B_{12} intake					
<9.4 mg/day	401 (50)	21.97 (12.22, 39.50)	0.04	6.92 (5.44, 8.80)	0.45
≥9.4 mg/day	401 (50)	36.19 (33.38, 39.24)		7.82 (7.44, 8.22)	
Folate and folic acid intake					
<600 μg/day	121 (15)	28.06 (22.92, 34.34)	0.02	7.22 (6.33, 8.23)	0.21
≥600 μg/day	681 (85)	37.38 (34.26, 40.78)		7.90 (7.50, 8.34)	
Maternal serum ferritin					
<15 μg/L	119 (15)	29.01 (23.68, 35.54)	0.02	7.00 (6.14, 7.98)	0.08
≥15 μg/L	613 (76)	38.00 (34.66, 41.67)		7.93 (7.50, 8.38)	

Note: N, sample size; BMI, Body mass index.
[a] p-value, comparing maternal urinary TAs and ∑As by different subgroups using Kruskal Wallis Test.
[b] Tobacco and alcohol consumption until the first trimester of pregnancy.

Table 2
Longitudinal studies on prenatal exposure to As and children's neuropsychological development.

Study (Country)	N pairs	As matrix	As species	Gestational age	Prenatal exposure levels (median [p25, p75]) μg/L	Age of children at test	Neurodevelopment test/scales		Effect
Present study (Spain)	807	Urine[a]	TAs AB ΣAs DMA MMA iAs		TAs: 32.6 (15.9, 71.8) ΣAs: 7.4 (4.6, 12.6) DMA: 6.6 (3.9, 11.3) MMA: 0.35 (0.23, 0.55) iAs: 0.32 (0.21, 0.53)	4–5 yrs	MSCA General scale Verbal scale Perceptive-performance scale Quantitative scale	Memory Motor scale Gross and fine motor scales Executive function Working memory	− for MMA concentrations and general, verbal, quantitative, memory, executive function and working memory scales. + for TAs and AB concentrations and verbal scale. − for %MMA
Vahter, 2020 (Bangladesh)	1460	Urine[b]	∑As	8 wg	82 (nd)	10 yrs	WISC-IV Verbal comprehension Perceptual reasoning Working memory	Processing speech Full development score	− for verbal comprehension, processing reasoning, processing speed and full development score. Worse effect in girls
Liang, 2020 (China)	2315	Cord serum	As	At delivery	1.89 (1.27, 2.89)	6 mo	ASQ • Communication • Gross motor • Fine motor	• Problem solving • Personal-social	> risk for problem solving and personal-social domains. Effect only in girls
Freire 2018 (Spain)	302	Placenta	As		<0.23 (nd) ng/g	4–5 yrs	MSCA General cognitive scaleMemory and memory span Motor function	Executive function (verbal and visual) Visual posterior cortex	− for global and verbal executive function scores (detectable As vs. non-detectable As).
Forns, 2014 (Spain)	385	Urine[a]	As	1st and 3rd trimester	1st T: 27.10 (13.40, 57.03) 3rd T: 29.80 (14.59,66.07)	4–5 yrs	MSCA General cognitive scale	Executive function	Ns for any scale.
Parajuli, 2013, 2014, 2015a, 2015b (Nepal)	74–100	Cord blood	As	At birth	1.46 (0.97, 1.74)	Next day after birth 6 mo 24mo	NBAS III Habituation, orientation, motor system, state organization, state regulation, autonomic stability, abnormal reflex.	BSID II Mental development Psychomotor development	At birth: − for state regulation. At 6 mo: Ns for any scale. At 24 mo: Ns for any scale.
Tofail, 2009 Hamadani, 2010, 2011 (Bangladesh)	1555 1700	Urine[b]	∑As	8 and 30 wg	8wg: 81 (24,380) [c] 30wg: 84 (26,415) [c]	7mo 18mo 5yrs	BSID II Psychomotor development PSTs Cognitive function	WPPSI Verbal intelligence quotient scale (VIQ) Full Scale Intelligence Quotient (FIQ)	At 7mo: Ns for any scale. At 18 mo: Ns for any scale. At 5 yrs: −for VIQ, all children at 8gw. Stratified analysis: − only for girls (8 and 30 wg) −for FIQ, all children at 8gw. Stratified analysis: − only for girls (8 and 30 wg)

TAs, Total As; ΣAs, sum of DMA, MMA and iAs; DMA, dimethylarsinic acid; MMA, monomethylarsonic acid; MSCA: the McCarthy Scales of Children's abilities; WISC-IV: Wechsler Intelligence Scale for Children; ASQ: the Ages and Stages Questionnaire; wg: weeks of gestation; yrs: years old; mo: months old; NBAS III: Brazelton neonatal behavioural assessment scale; BSID II: Bayley Scales of Infant Development; PSTs: problem solving test; WPPSI: Wechsler Preschool and Primary Scale of Intelligence.

[a] Adjusted for creatinine (μg/gram creatinine).
[b] Adjusted for specific gravity.
[c] Median (p10, p90).

growing number of studies have shown that the intermediate compounds, especially forms in the trivalent oxidation state (MMA^{III} and DMA^{III}) could have higher reactivity and toxicity (Mass et al., 2001; Nesnow et al., 2002; Styblo et al., 2000). In fact, increasing %MMA has been found to be associated with a higher risk of several adverse health effects, such as lung and skin cancer, atherosclerosis and cardiovascular diseases (Gamboa-Loira et al., 2017; Kuo et al., 2017; Tseng, 2007). In our study, we observed an inverse association between %MMA and the scores on the memory scale. Another association found in our study was a positive relationship between %iAs and the score on the gross motor scale. A possible explanation is that a higher %iAs is indicating a lower proportion of %MMA or %DMA. However, when the PCs were used as methylation phenotypes, with the purpose of preventing the high correlation between the three metabolite percentages, no association was observed.

We have also noted a positive association between both TAs and AB concentrations and scores for the verbal scale. Fish and seafood consumption is the main source of the organic form AB (European Food Safety Authority, 2009), and is the highest contributor to the total As concentrations. Even though fish consumption is a source of toxicants (i.e. As, but also methylmercury and polychlorinated biphenyls), it also provides some essential nutrients related to better neurodevelopment, such as proteins, vitamin D and, especially, n-3 long-chain polyunsaturated fatty acids (LCPUFA) (FAO/WHO, 2010; Julvez et al., 2016). Although the models were adjusted for fish consumption, it is possible that the food frequency questionnaire did not fully record the maternal fish intake variability, thereby introducing some degree of misclassification.

In our study, we have shown an interaction between As metabolism and Fe status. Children whose mothers presented increasing %iAs and ID (ferritin <15 μg/L) obtained lower scores on the general, verbal and memory scales. Several epidemiological studies have analysed the relationship between As exposure and its metabolism and Fe status. In a cross-sectional study conducted on children, ID was associated with lower %iAs and higher %DMA (Kordas et al., 2016). Conversely, another study carried out with Mexican children showed associations between higher serum ferritin concentration and lower %MMA and higher % DMA (Kordas et al., 2017). The authors of this study suggested that a better Fe status may promote a more efficient arsenic methylation. In a cohort of pregnant Bangladeshi women, Li et al. (2008) found no association between serum ferritin levels and any As metabolite percentage. Fe has been used as an efficient chelator agent for removal of As in water, due to its ability to bind to arsenic and transform the soluble As into insoluble compounds (Flora, 2015). Nevertheless, the relationship between arsenic and Fe in the human body remains unclear. In experimental studies, Fe concentrations seem to diminish the bioaccessibility of As in the gut and increase its methylation (Clemente et al., 2019; Yu et al., 2016). Liu et al. (2013) also showed that the decrease in the bioavailability of As due to Fe co-exposure resulted in a decrease in the toxicity of As in an animal experiment. Nevertheless, the interactive effects of As exposure and its metabolism and the Fe status should continue to be studied.

Finally, we have found that the relationship between As methylation efficiency and scores on the MSCA sub-scales was modified by maternal urinary Zn. Children whose mother had Zn levels below 363.7 μg/L obtained lower scores on the three motor scales with increasing %MMA. Few epidemiological studies have analysed the interactive effect between As and Zn. Most of them have focused on the effect of Zn on As metabolism. For example, in pregnant Bangladeshi women, plasma Zn was associated with higher %MMA and iAs and lower %DMA, only at the highest level of As exposure (Li et al., 2008). Conversely, in a study carried out with Mexican children, neither basal Zn concentration nor Zn supplementation modified As metabolism (Kordas et al., 2017). Several experimental studies have investigated the possible role of Zn in As concentrations and toxicity. The administration of Zn in As-exposed rodent models restored glutathione levels, which were diminished by As exposure, decreased lipid peroxidase concentrations and increased induction of metallothionein (Ganger et al., 2016; Kumar et al., 2010). These mechanisms seem to protect against As toxicity. Similarly, jointly increasing As concentrations and Zn deficiency induced oxidative stress in cell models (Wong et al., 2019). We also evaluated children's sex as a possible modifying factor and observed differences in the coefficients between girls and boys; however, the interaction p-values were not statistically significant.

Apart from the influence of some nutrients and elements on As neurotoxicity, the association between prenatal MMA concentrations and the MSCA scales observed in our study could be reflecting the relationship between other variables with an influence on As metabolism, such as maternal smoking and the OCM nutrients. Some studies have shown an association between smoking during pregnancy and OCM nutrients; women smokers presented lower folate and vitamin B_{12} levels and higher homocysteine levels (Tuenter et al., 2019) than non-smokers. Nevertheless, we have analysed this possible confounder through two approaches: 1) adjusting the main models with the maternal tobacco consumption during the first trimester of pregnancy, and 2) evaluating the smoking habit as a potential modifier by interaction terms. In both approaches, we did not observe any influence of the maternal smoking habit on the inverse association between MMA concentrations and the MSCA subscales (data not shown). However, due to the complexity of the interrelations between As metabolism, micronutrients and other factors, such as genetic factors, the interpretation of the association of MMA concentrations and child neurodevelopment should be taken with caution.

This study has several limitations. Firstly, a considerable proportion of children from the cohort were not included in the present study and this loss to follow-up could represent a selection bias. We observed that the participants who were included had a higher socioeconomic profile and educational level. This fact could be affecting the representativity of the study and the estimation of some exposure-outcome associations. Another limitation is the assessment of As exposure at only one timepoint during pregnancy. As the vulnerability of the central nervous system extends from the beginning of pregnancy to adolescence, and As metabolism efficiency seems to change during pregnancy, it would have been more accurate to measure As and its metabolites at several timepoints during pregnancy, as well as in the postnatal period. In the present work, multiple analyses were performed, particularly in the interaction analysis, therefore results should be taken with caution because some significant associations could result from chance. Coefficients and their confidence intervals should be taken as a global representation of the pattern of the relations between the variables involved in the study (Rothman, 1990). Finally, although we evaluated multiple potential confounders, the present study lacks information on some important variables that could have an influence on cognitive development (such as the postnatal growth environment), and on the relationship between As methylation and child neurodevelopment, such as other nutrients involved in one-carbon metabolism (i.e. choline and betaine) and genetic information. For this reason, we cannot rule out the possibility of residual confounding in the relationships between As exposure and its metabolism and the results of neuropsychological development.

The major strength of our study is its prospective and extended assessment of information, as this enabled us to obtain sufficient information about characteristics of the mothers and the children, including levels of other metals and essential elements, and estimated dietary nutrients, which may affect both As exposure and its metabolism and neuropsychological development. Another strength is the analysis of As speciation in a considerably large sample. As far as we know, this is one of the largest European studies to have analysed the association of prenatal As species concentrations and methylation efficiency with child neurodevelopment. Finally, another advantage is the analysis of As methylation efficiency through two approaches: using the relative percentages of each As metabolite and principal component analysis.

5. Conclusions

We have observed an inverse association between prenatal MMA concentrations and children's neuropsychological development at 4–5 years of age, specifically in verbal, quantitative, executive function and working memory development. Furthermore, evidence has been found of an inverse relationship between prenatal %MMA and memory function. Lower maternal Mn, Zn and ferritin concentrations seem to influence the relationship between As methylation and child neurodevelopment. Children whose mothers had lower concentrations of these nutrients and elements obtained lower scores with decreasing As methylation efficiency.

As exposure and its metabolism is a complex process that is not yet fully understood, and the possible toxics effects on the neuropsychological development could be entangled with several factors that exert an influence on this relationship. Owing to these reasons, the results presented in this research should be interpreted with caution. Similar epidemiological studies are necessary in order to improve knowledge about exposure to arsenic, as well as its different species, during critical periods such as prenatal development and its effects on children's health. Also, in combination with more well-designed epidemiological studies, there is a need for studies that can provide mechanistic data of As neurotoxicity. It is also necessary to investigate further in order to understand the impact of As methylation during pregnancy and the possible interaction with several factors. Altogether, this knowledge could be used to propose new recommendations and public health strategies.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We are grateful to all the participants and their families for taking part in this study. We also appreciate the generous contribution made to the study by all the members of INMA. A full roster of the INMA Project Investigators can be found at http://www.proyectoinma.org/presentacion-inma/listado-investigadores/en_listado-investigadores.html. This study was supported by grants from Instituto de Salud Carlos III [FIS-FEDER: 06/0867, 07/0314; FIS 11/01007, 13/1944, 16/1288, 18/01142, 19/1338; Miguel Servet- FEDER: CP15/0025, CPII20/00006; FIS-FSE: 17/00260; Miguel Servet-FSE: MS15/0025, MS20/0006incl. FEDER funds], Generalitat Valenciana [BEST/2020/059and AICO/2020/285], Department of Health of the Basque Government [2005111093], Provincial Government of Gipuzkoa [DFG06/002], and annual agreements with municipalities in the study area (Zumarraga, Urretxu, Legazpi, Azkoitia, Azpeitia and Beasain).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2021.112208.

Policy and ethics

The study protocol was approved by the Ethics Committee of the university hospital La Fe (Valencia), the Ethics Committee of the Public Health Research Centre in Valencia (CSISP) and the Ethics Committee of Donostia Hospital (Gipuzkoa). Informed consent was obtained from all participants in each phase.

Funding sources

This study was supported by grants from Instituto de Salud Carlos III [FIS-FEDER: 06/0867, 07/0314; FIS 11/01007, 13/1944, 16/1288, 18/01142, 19/1338; Miguel Servet- FEDER: CP15/0025, FIS-FSE: 17/00260; Miguel Servet-FSE: MS15/0025, MSII20/0006 incl. FEDER funds], Generalitat Valenciana [BEST/2020/059 and AICO/2020/285], Department of Health of the Basque Government [2,005,111,093], Provincial Government of Gipuzkoa [DFG06/002] and annual agreements with municipalities in the study area (Zumarraga, Urretxu, Legazpi, Azkoitia, Azpeitia and Beasain).

References

Agence nationale de sécurité sanitaire de l'alimentation de l'environnement et du travail, 2011. Second French Total Diet Study. ANSES Opinion.

Agència Catalana de Seguretat Alimentaria, 2020. Contaminants químics . V Estudi de dieta total a Catalunya Metalls pesants , dioxines (PCDD/F) i bifenils policlorats (PCB). Generalitat de Catalunya. Departament de Salut.

Arija, V., Hernández-Martínez, C., Tous, M., Canals, J., Guxens, M., Fernández-Barrés, S., Ibarluzea, J., Izaro, B., Soler-Blasco, R., Llop, S., Vioque, J., Sunyer, J., Julvez, J., 2019. Association of iron status and intake during pregnancy with neuropsychological outcomes in children aged 7 Years: the prospective birth cohort Infancia y Medio Ambiente (INMA) study. Nutrients 11. https://doi.org/10.3390/nu11122999.

Challenger, F., 1945. Biological methylation. Chem. Rev. 36, 315–361. https://doi.org/10.1021/cr60115a003.

Chandravanshi, L.P., Gupta, R., Shukla, R.K., 2018. Developmental neurotoxicity of arsenic: involvement of oxidative stress and mitochondrial functions. Biol. Trace Elem. Res. 186, 185–198. https://doi.org/10.1007/s12011-018-1286-1.

Chandravanshi, L.P., Yadav, R.S., Shukla, R.K., Singh, A., Sultana, S., Pant, A.B., Parmar, D., Khanna, V.K., 2014. Reversibility of changes in brain cholinergic receptors and acetylcholinesterase activity in rats following early life arsenic exposure. Int. J. Dev. Neurosci. 34, 60–75. https://doi.org/10.1016/j.ijdevneu.2014.01.007.

Clemente, M.J., Cimbalo, A., Chiocchetti, G., Devesa, V., Vélez, D., 2019. Dietary compounds to reduce in vivo inorganic arsenic bioavailability. J. Agric. Food Chem. 67, 9032–9038. https://doi.org/10.1021/acs.jafc.9b03372.

European Food Safety Authority, 2014. Dietary exposure to inorganic arsenic in the European population. Eur. Food Saf. Auth. J. 12 https://doi.org/10.2903/j.efsa.2014.3597.

European Food Safety Authority, 2009. Scientific Opinion on arsenic in food. Eur. Food Saf. Auth. J. 7 https://doi.org/10.2903/j.efsa.2009.1351.

FAO/WHO, 2010. Report of the joint FAO/WHO expert consultation on the risks and benefits of fish consumption. FAO Fisheries and Acquaculture report. WHO, Rome.

Flora, S.S., 2015. Arsenic: chemistry, occurrence, and exposure. In: Flora, S.S. (Ed.), Handbook of Arsenic Toxicology. Elsevier, Amsterdam.

Forns, J., Fort, M., Casas, M., Cáceres, A., Guxens, M., Gascon, M., Garcia-Esteban, R., Julvez, J., Grimalt, J.O., Sunyer, J., 2014. Exposure to metals during pregnancy and neuropsychological development at the age of 4 years. Neurotoxicology 40, 16–22. https://doi.org/10.1016/J.NEURO.2013.10.006.

Freire, C., Amaya, E., Gil, F., Fernández, M.F., Murcia, M., Llop, S., Andiarena, A., Aurrekoetxea, J., Bustamante, M., Guxens, M., Ezama, E., Fernández-Tardón, G., Olea, N., 2018. Prenatal co-exposure to neurotoxic metals and neurodevelopment in preschool children: the Environment and Childhood (INMA) Project. Sci. Total Environ. 621, 340–351. https://doi.org/10.1016/j.scitotenv.2017.11.273.

Gamboa-Loira, B., Cebrián, M.E., Franco-marina, F., López-carrillo, L., 2017. Arsenic metabolism and cancer risk : a meta-analysis. Environ. Res. 156, 551–558. https://doi.org/10.1016/j.envres.2017.04.016.

Ganger, R., Garla, R., Mohanty, B.P., Bansal, M.P., Garg, M.L., 2016. Protective effects of zinc against acute arsenic toxicity by regulating antioxidant defense system and cumulative metallothionein expression. Biol. Trace Elem. Res. 169, 218–229. https://doi.org/10.1007/s12011-015-0400-x.

Gardner, R.M., Nermell, B., Kippler, M., Grandér, M., Li, L., Ekström, E.C., Rahman, A., Lönnerdal, B., Hoque, A.M.W., Vahter, M., 2011. Arsenic methylation efficiency increases during the first trimester of pregnancy independent of folate status. Reprod. Toxicol. 31, 210–218. https://doi.org/10.1016/j.reprotox.2010.11.002.

Georgescu, I.A., Popa, D., Zagrean, L., 2020. The anatomical and functional heterogeneity of the mediodorsal thalamus. Brain Sci. 10, 1–17. https://doi.org/10.3390/brainsci10090624.

Grandjean, P., Landrigan, P.J., 2006. Developmental neurotoxicity of industrial chemicals. Lancet 368, 2167–2178. https://doi.org/10.1016/S0140-6736(06)69665-7.

Guxens, M., Ballester, F., Espada, M., Ferna, M.F., Grimalt, J.O., Ibarluzea, J., Olea, N., Rebagliato, M., Tardón, A., Torrent, M., Vioque, J., Vrijheis, M., Sunyer, J., INMA project, 2012. Cohort profile : the INMA — INfancia y Medio Ambiente —(environment and childhood) Project. Int. J. Epidemiol. 41, 930–940. https://doi.org/10.1093/ije/dyr054.

Hall, M., Gamble, M., Slavkovich, V., Liu, X., Levy, D., Cheng, Z., van Geen, A., Yunus, M., Rahman, M., Pilsner, J.R., Graziano, J., 2007. Determinants of arsenic metabolism: blood arsenic metabolites, plasma folate, cobalamin, and homocysteine

concentrations in maternal-newborn pairs. Environ. Health Perspect. 115, 1503–1509. https://doi.org/10.1289/ehp.9906.
Hamadani, J., Tofail, F., Nermell, B., Gardner, R., Shiraji, S., Bottai, M., Arifeen, S., Huda, S., Vahter, M., 2011. Critical windows of exposure for arsenic-associated impairment of cognitive function in pre-school girls and boys: a population-based cohort study. Int. J. Epidemiol. 40, 1593–1604. https://doi.org/10.1093/ije/dyr176.
Hayakawa, T., Kobayashi, Y., Cui, X., 2005. A new metabolic pathway of arsenite : arsenic – glutathione complexes are substrates for human arsenic methyltransferase Cyt 19. Arch. Toxicol. 79, 183–191. https://doi.org/10.1007/s00204-004-0620-x.
Hopenhayn, C., Huang, B., Christian, J., Peralta, C., Ferreccio, C., Atallah, R., Kalman, D., 2003. Profile of urinary arsenic metabolites during pregnancy. Environ. Health Perspect. 111, 1888–1891. https://doi.org/10.1289/ehp.6254.
Hsieh, R.L., Huang, Y.L., Shiue, H.S., Huang, S.R., Lin, M.I., Mu, S.C., Chung, C.J., Hsueh, Y.M., 2014. Arsenic methylation capacity and developmental delay in preschool children in Taiwan. Int. J. Hyg Environ. Health 217, 678–686. https://doi.org/10.1016/j.ijheh.2014.02.004.
Iniguez, C., Ballester, F., Estarlich, M., Llop, S., Fernandez-Patier, R., Aguirre-Alfaro, A., Esplugues, A., 2009. Estimation of personal NO2 exposure in a cohort of pregnant women. Sci. Total Environ. 407, 6093–6099. https://doi.org/10.1016/j.scitotenv.2009.08.006.
Jones, M.R., Tellez-Plaza, M., Vaidya, D., Grau, M., Francesconi, K.A., Goessler, W., Guallar, E., Post, W.S., Kaufman, J.D., Navas-Acien, A., 2016. Estimation of inorganic arsenic exposure in populations with frequent seafood intake: evidence from MESA and NHANES. Am. J. Epidemiol. 184, 590–602. https://doi.org/10.1093/aje/kww097.
Julvez, J., Forns, M., Ribas-Fitó, N., Torrent, M., Sunyer, J., 2011. Attention behavior and hyperactivity and concurrent neurocognitive and social competence functioning in 4-year-olds from two population-based birth cohorts. Eur. Psychiatry 26, 381–389. https://doi.org/10.1016/j.eurpsy.2010.03.013.
Julvez, J., Méndez, M., Fernandez-Barres, S., Romaguera, D., Vioque, J., Llop, S., Ibarluzea, J., Guxens, M., Avella-Garcia, C., Tardón, A., Riaño, I., Andiarena, A., Robinson, O., Arija, V., Esnaola, M., Ballester, F., Sunyer, J., 2016. Maternal consumption of seafood in pregnancy and child neuropsychological development: a longitudinal study based on a population with high consumption levels. Am. J. Epidemiol. 183, 169–182. https://doi.org/10.1093/aje/kwv195.
Kordas, K., Queirolo, E.I., Peregalli, F., Hsiao, P.Y., Lu, Y., Vahter, M., Institutet, K., Mañay, N., Peregalli, F., Hsiao, P.Y., Lu, Y., Vahter, M., 2016. Low-level arsenic exposure: nutritional and dietary predictors in first-grade Uruguayan children. Environ. Res. 147, 16–23. https://doi.org/10.1016/j.envres.2016.01.022.
Kordas, K., Roy, A., López, P., García-Vargas, G., Cebrián, M.E., Vera-Aguilar, E., Rosado, J.L., 2017. Iron and zinc supplementation does not impact urinary arsenic excretion in Mexican school children. J. Pediatr. 185, 205–210. https://doi.org/10.1016/j.jpeds.2017.02.040 e1.
Kumar, A., Malhotra, A., Nair, P., Garg, M.L., Dhawan, D.K., 2010. Protective role of zinc in ameliorating arsenic-induced oxidative stress and histological changes in rat liver. J. Environ. Pathol. Toxicol. Oncol. 29, 91–100. https://doi.org/10.1615/JEnvironPatholToxicolOncol.v29.i2.30.
Kuo, C., Moon, K.A., Wang, S., Silbergeld, E., Navas-acien, A., 2017. The association of arsenic metabolism with cancer , cardiovascular disease , and diabetes : a systematic review of the epidemiological evidence. Environ. Health Perspect. 125.
Kurzius-Spencer, M., Thomson, C.A., Hartz, V., Hsu, C., Burgess, J.L., Kay, M., Rourke, O., Harris, R.B., 2017. Nutrients in one-carbon metabolism and urinary arsenic methylation in the national health and nutrition examination survey (NHANES) 2003–2004. Sci. Total Environ. 381–390. https://doi.org/10.1016/j.scitotenv.2017.07.019, 607–608.
Laine, J.E., Ilievski, V., Richardson, D.B., Herring, A.H., Stýblo, M., Rubio-Andrade, M., Garcia-Vargas, G., Gamble, M.V., Fry, R.C., 2018. Maternal one carbon metabolism and arsenic methylation in a pregnancy cohort in Mexico. J. Expo. Sci. Environ. Epidemiol. 28, 505–514. https://doi.org/10.1038/s41370-018-0041-1.
Lee, P.H., 2014. Is a cutoff of 10% appropriate for the change-in-estimate criterion of confounder identification? J. Epidemiol. 24, 161–167. https://doi.org/10.2188/jea.JE20130062.
Li, J., Guo, Y., Duan, X., Li, B., 2020. Tissue- and region-specific accumulation of arsenic species, especially in the brain of mice, after long-term arsenite exposure in drinking water. Biol. Trace Elem. Res. 198, 168–176. https://doi.org/10.1007/s12011-020-02033-x.
Li, L., Ekström, E.C., Goessler, W., Lönnerdal, B., Nermell, B., Yunus, M., Rahman, A., El Arifeen, S., Persson, L.Å., Vahter, M., 2008. Nutritional status has marginal influence on the metabolism of inorganic arsenic in pregnant Bangladeshi women. Environ. Health Perspect. 116, 315–321. https://doi.org/10.1289/ehp.10639.
Liang, C., Wu, X., Huang, K., Yan, S., Li, Z., Xia, X., Pan, W., Sheng, J., Tao, R., Tao, Y., Xiang, H., Hao, J., Wang, Q., Tong, S., Tao, F., 2020. Domain- and sex-specific effects of prenatal exposure to low levels of arsenic on children's development at 6 months of age: findings from the Ma'anshan birth cohort study in China. Environ. Int. 135, 105112. https://doi.org/10.1016/j.envint.2019.105112.
Liu, S., Guo, X., Zhang, X., Cui, Y., Zhang, Y., Wu, B., 2013. Impact of iron precipitant on toxicity of arsenic in water: a combined in vivo and in vitro study. Environ. Sci. Technol. 47, 3432–3438. https://doi.org/10.1021/es400176m.
López-Carrillo, L., Gamboa-Loira, B., Becerra, W., Hernández-Alcaraz, C., Hernández-Ramírez, R., Gandolfi, A., Cebrián, M., 2016. Dietary micronutrient intake and its relationship with arsenic metabolism in Mexican women. Environ. Res. 151, 445–450. https://doi.org/10.1016/j.envres.2016.08.015 (Dietary).
Lozano, M., Murcia, M., Soler-Blasco, R., Iñiguez, C., Irizar, A., Lertxundi, A., Basterrechea, M., Marina, L.S., Amorós, R., Broberg, K., Ballester, F., Llop, S., 2020. Prenatal Se concentrations and anthropometry at birth in the INMA study (Spain). Environ. Res. https://doi.org/10.1016/j.envres.2019.108943, 108943.
Luo, J., Shu, W., 2015. Arsenic-induced developmental neurotoxicity. In: Flora, S.S. (Ed.), Hanbook of Arsenic Toxicology. Elsevier, Amsterdam.
Mass, M.J., Tennant, A., Roop, B.C., Cullen, W.R., Styblo, M., Thomas, D.J., Kligerman, A.D., 2001. Methylated trivalent arsenic species are genotoxic. Chem. Res. Toxicol. 14, 355–361. https://doi.org/10.1021/tx000251l.
McCarthy, D., 2009. MSCA. Escalas McCarthy de Aptitudes y Psicomotricidad para Niños. TEA Ediciones, Madrid.
Ministerio de Sanidad Servicios Sociales e Igualdad, 2019. Calidad del Agua de Consumo Humano en España 2019. Anexo: Tablas. Ministerio de sanidad servicios sociales e igualdad, Madrid.
Mishra, D., Flora, S., 2008. Differential oxidative stress and DNA damage in rat brain regions and blood following chronic arsenic exposure. Toxicol. Ind. Health 24, 247–256. https://doi.org/10.1177/0748233708093355.
Molin, M., Ulven, S., Dahl, L., Telle-Hansen, V., Holck, M., Skjegstad, G., Ledsaak, O., Sloth, J.J., Goessler, W., Oshaug, A., Alexander, J., Fliegel, D., Ydersbond, T.A., Meltzer, H.M., 2012. Humans seem to produce arsenobetaine and dimethylarsinate after a bolus dose of seafood. Environ. Res. 112, 28–39. https://doi.org/10.1016/j.envres.2011.11.007.
Navas-Acien, A., Francesconi, K.A., Silbergeld, E.K., Guallar, E., 2011. Seafood intake and urine concentrations of total arsenic, dimethylarsinate and arsenobetaine in the US population. Environ. Res. 111, 110–118. https://doi.org/10.1016/j.envres.2010.10.009.
Nesnow, S., Roop, B.C., Lambert, G., Kadiiska, M., Mason, R.P., Cullen, W.R., Mass, M.J., 2002. DNA damage induced by methylated trivalent arsenicals is mediated by reactive oxygen species. Chem. Res. Toxicol. 15, 1627–1634. https://doi.org/10.1021/tx025598y.
Nordberg, G.F., Jin, T., Hong, F., Zhang, A., Buchet, J.P., Bernard, A., 2005. Biomarkers of cadmium and arsenic interactions. Toxicol. Appl. Pharmacol. 206, 191–197. https://doi.org/10.1016/J.TAAP.2004.11.028.
Palma, I., Farran, P., Cervera, P., 2008. Tables of Food Composition for Household Measures of Habitual Consumption in Spain: CESNID, fourth ed. McGraw Hill, Madrid.
Parajuli, R.P., Fujiwara, T., Umezaki, M., Furusawa, H., Watanabe, C., 2014. Home environment and prenatal exposure to lead , arsenic and zinc on the neurodevelopment of six-month-old infants living in. Neurotoxicol. Teratol. 41, 89–95. https://doi.org/10.1016/j.ntt.2013.12.006.
Parajuli, R.P., Fujiwara, T., Umezaki, M., Watanabe, C., 2015a. Home environment and cord blood levels of lead, arsenic, and zinc on neurodevelopment of 24 months children living in Chitwan Valley, Nepal. J. Trace Elem. Med. Biol. 29, 315–320. https://doi.org/10.1016/J.JTEMB.2014.08.006.
Parajuli, R.P., Fujiwara, T., Umezaki, M., Watanabe, C., 2013. Association of cord blood levels of lead, arsenic, and zinc with neurodevelopmental indicators in newborns: a birth cohort study in Chitwan Valley, Nepal. Environ. Res. 121, 45–51. https://doi.org/10.1016/J.ENVRES.2012.10.010.
Parajuli, R.P., Umezaki, M., Fujiwara, T., Watanabe, C., 2015b. Association of cord blood levels of lead, arsenic, and zinc and home environment with children neurodevelopment at 36 Months living in chitwan valley, Nepal. PLoS One 10, e0120992. https://doi.org/10.1371/journal.pone.0120992.
Piao, F., Li, S., Guan, H., 2015. Developmental arsenic exposure impacts fetal Programming of the nervous system. In: Flora, S.S. (Ed.), Hanbook of Arsenic Toxicology. Elsevier, Amsterdam.
Punshon, T., Davis, M.A., Marsit, C.J., Theiler, S.K., Baker, E.R., Jackson, B.P., Conway, D.C., Karagas, M.R., 2015. Placental arsenic concentrations in relation to both maternal and infant biomarkers of exposure in a US cohort. J. Expo. Sci. Environ. Epidemiol. 25, 599–603. https://doi.org/10.1038/jes.2015.16.
R Core Team, 2020. R: A Language and Environment for Statistical Computing.
Rahman, M.M., Hossain, K.F.B., Banik, S., Sikder, M.T., Akter, M., Bondad, S.E.C., Rahaman, M.S., Hosokawa, T., Saito, T., Kurasaki, M., 2019. Selenium and zinc protections against metal-(loids)-induced toxicity and disease manifestations: a review. Ecotoxicol. Environ. Saf. 168, 146–163. https://doi.org/10.1016/j.ecoenv.2018.10.054.
Rosado, J.L., Ronquillo, D., Kordas, K., Rojas, O., Alatorre, J., Lopez, P., Garcia-Vargas, G., del Carmen Caamaño, M., Cebrián, M.E., Stoltzfus, R.J., 2007. Arsenic exposure and cognitive performance in Mexican Schoolchildren. Environ. Health Perspect. 115, 1371–1375. https://doi.org/10.1289/ehp.9961.
Rothman, K., 1990. No adjustments are needed for multiple comparisons. Epidemiology 1, 43–46.
Sánchez-Peña, L.C., Petrosyan, P., Morales, M., González, N.B., Gutiérrez-Ospina, G., Del Razo, L.M., Gonsebatt, M.E., 2010. Arsenic species, AS3MT amount, and AS3MT gen expression in different brain regions of mouse exposed to arsenite. Environ. Res. 110, 428–434. https://doi.org/10.1016/j.envres.2010.01.007.
Sanchez, T.R., Perzanowski, M., Graziano, J.H., 2016. Inorganic arsenic and respiratory health, from early life exposure to sex-specific effects: a systematic review. Environ. Res. 147, 537–555. https://doi.org/10.1016/j.envres.2016.02.009.
Shahid, N.M., Imran, M., Khalid, S., BehzadNabeel, Murtaza, Khan Niazi, B., Zhang, Y., Hussain, I., Natasha, Shahid, Muhammad Imran, M., Khalid, S., Murtaza, B., Khan Niazi, N., Zhang, Y., Hussain, I., 2020. Arsenic environmental contamination status in South Asia. In: Srivastava, S. (Ed.), Arsenic in Drinking Water and Food. Springer Nature Singapore, Singapore.
Siddiqui, S., Chatterjee, U., Kumar, D., Siddiqui, A., Goyal, N., 2008. Neuropsychology of prefrontal cortex. Indian J. Psychiatry 50, 202. https://doi.org/10.4103/0019-5545.43634.
Soler-Blasco, R., Murcia, M., Lozano, M., Esplugues, A., Vioque, J., Lertxundi, N., Santa Marina, L., Lertxundi, A., Irizar, A., Braeuer, S., Goesler, W., Ballester, F., Llop, S.,

2021. Urinary arsenic species and methylation efficiency during pregnancy: concentrations and associated factors in Spanish pregnant women. Environ. Res. 110889. https://doi.org/10.1016/j.envres.2021.110889.

Soler-Blasco, R., Murcia, M., Lozano, M., González-Safont, L., Amorós, R., Ibarluzea, J., Broberg, K., Irizar, A., Lopez-Espinosa, M., Lertxundi, N., Santa Marina, L., Ballester, F., Llop, S., 2020. Prenatal manganese exposure and neuropsychological development in early childhood in the INMA cohort. Int. J. Hyg Environ. Health 224, 113443. https://doi.org/10.1016/j.ijheh.2019.113443.

Stiles, J., Jernigan, T.L., 2010. The basics of brain development. https://doi.org/10.1007/s11065-010-9148-4, 327, 348.

Styblo, M., Del Razo, L.M., Vega, L., Germolec, D.R., LeCluyse, E.L., Hamilton, G.A., Reed, W., Wang, C., Cullen, W.R., Thomas, D.J., 2000. Comparative toxicity of trivalent and pentavalent inorganic and methylated arsenicals in rat and human cells. Arch. Toxicol. 74, 289–299. https://doi.org/10.1007/s002040000134.

Taylor, V., Goodale, B., Raab, A., Schwerdtle, T., Reimer, K., Conklin, S., Karagas, M.R., Francesconi, K.A., 2017. Human exposure to organic arsenic species from seafood. Sci. Total Environ. 15, 266–282. https://doi.org/10.1016/j.scitotenv.2016.12.113. Human.

Tolins, M., Ruchirawat, M., Landrigan, P., 2014. The developmental neurotoxicity of arsenic: cognitive and behavioral consequences of early life exposure. Ann. Glob. Heal. 80, 303–314. https://doi.org/10.1016/J.AOGH.2014.09.005.

Trasande, L., Niu, J., Li, J., Liu, X., Zhang, B., Li, Z., Ding, G., Sun, Y., Chen, M., Hu, X., Chen, L., Mendelsohn, A., Chen, Y., Qu, Q., 2014. The environment and children's health care in Northwest China. BMC Pediatr. 14, 1–8.

Tseng, C.H., 2007. Arsenic methylation, urinary arsenic metabolites and human diseases: current perspective. J. Environ. Sci. Health Part C Environ. Carcinog. Ecotoxicol. Rev. 25, 1–22. https://doi.org/10.1080/10590500701201695.

Tuenter, A., Bautista Nino, P.K., Vitezova, A., Pantavos, A., Bramer, W.M., Franco, O.H., Felix, J.F., 2019. Folate, vitamin B12, and homocysteine in smoking-exposed pregnant women: a systematic review. Matern. Child Nutr. 15 https://doi.org/10.1111/mcn.12675.

Tyler, C.R., Allan, A.M., 2014. The effects of arsenic exposure on neurological and cognitive dysfunction in human and rodent studies: a review. Curr. Environ. Heal. reports 1, 132–147. https://doi.org/10.1007/s40572-014-0012-1.

U.S. Department of Agriculture: Agricultural Research Service USDA, 2007. National Nutrient Database for Standard Reference, Release 25. [WWW Document]. URL https://www.ars.usda.gov/ARSUserFiles/80400525/Data/SR25/sr25_doc.pdf.

Vahter, M., Skröder, H., Rahman, S.M., Levi, M., Derakhshani Hamadani, J., Kippler, M., 2020. Prenatal and childhood arsenic exposure through drinking water and food and cognitive abilities at 10 years of age: a prospective cohort study. Environ. Int. 139, 105723. https://doi.org/10.1016/j.envint.2020.105723.

Vahter, M.E., 1999. Methylation of inorganic arsenic in different mammalian species and population groups. Sci. Prog. 82, 69–88. https://doi.org/10.1177/003685049908200104.

Valeri, L., Mazumdar, M.M., Bobb, J.F., Henn, B.C., Rodrigues, E., Sharif, O.I.A., Kile, M. L., Quamruzzaman, Q., Afroz, S., Golam, M., Amarasiriwardena, C., Bellinger, D.C., Christiani, D.C., Coull, B.A., Wright, R.O., 2016. The joint effect of prenatal exposure to metal mixtures on neurodevelopmental outcomes at 20 – 40 Months of age : evidence from rural Bangladesh. Environ. Health 125, 067015. https://doi.org/10.1289/EHP614.

Vioque, J., Navarrete-Muñoz, E.-M., Gimenez-Monzó, D., García-de-la-Hera, M., Granado, F., Young, I.S., Ramón, R., Ballester, F., Murcia, M., Rebagliato, M., Iñiguez, C., 2013. Reproducibility and validity of a food frequency questionnaire among pregnant women in a Mediterranean area. Nutr. J. 12, 26. https://doi.org/10.1186/1475-2891-12-26.

Willet, W., 2013. Nutritional Epidemiology. Oxford University Press, Oxford.

Wong, C.P., Dashner-titus, E.J., Alvarez, S.C., Chase, T.T., Hudson, L.G., Ho, E., 2019. Zinc deficiency and arsenic exposure can act both independently or cooperatively to affect zinc status, oxidative stress, and inflammatory response Carmen. Biol. Trace Elem. Res. 191, 370–381. https://doi.org/10.1007/s12011-019-1631-z.Zinc.

World Health Organization, 2017. Guidelines for Drinking-Water Quality, fourth ed. World Health Organization, Geneva.

Yu, H., Wu, B., Zhang, X.X., Liu, S., Yu, J., Cheng, S., Ren, H.Q., Ye, L., 2016. Arsenic metabolism and toxicity influenced by ferric iron in simulated gastrointestinal tract and the roles of gut microbiota. Environ. Sci. Technol. 50, 7189–7197. https://doi.org/10.1021/acs.est.6b01533.

Zhong, Q., Cui, Y., Wu, H., Niu, Q., Lu, X., Wang, L., Huang, F., 2019. Association of maternal arsenic exposure with birth size: a systematic review and meta-analysis. Environ. Toxicol. Pharmacol. 69, 129–136. https://doi.org/10.1016/j.etap.2019.04.007.

CAPÍTULO VI. DISCUSIÓN GENERAL

6.1DISCUSIÓN GENERAL

6.1.1 Niveles prenatales de As y Mn (artículo I y II)

Los niveles de Mn en suero descritos en el **artículo I** de la presente tesis fueron más bajos que los informados en otros trabajos donde las participantes también fueron mujeres embarazadas. Por ejemplo, en el estudio de Yu et.al., (2013), llevado a cabo en China, la mediana de las concentraciones en suero materno fue de 2,8 µg/L. Estos niveles fueron muy similares a los observados en un estudio realizado en Suecia, donde la mediana de Mn en la misma matriz fue de 2,7 µg/L (Ode et al., 2015). Estas diferencias podrían deberse, en parte, al momento del desarrollo prenatal en el que se recogieron las muestras (o del muestreo). Por ejemplo, en los estudios comentados anteriormente, los niveles de Mn se analizaron en muestras recogidas en el momento del parto, mientras que, en nuestro estudio, se midieron en muestras de suero materno recogidas durante el primer trimestre del embarazo. Igualmente, en el estudio de Zhou et.al., (2021) la mediana de las concentraciones en suero materno recogido durante el primer trimestre del embarazo, como en nuestro estudio, fueron menores (0,85 µg/L). Se ha descrito una tendencia creciente de las concentraciones de Mn a lo largo del embarazo (Kupsco et al., 2020; Spencer, 1999; Takser et al., 2004), probablemente debido a una mayor absorción intestinal de este elemento, asociado a la deficiencia de hierro fisiológica presente durante este periodo (Abbassi-Ghanavati et al., 2009; Finley, 1999). Además del momento de muestreo, la variabilidad en la matriz empleada para medir las concentraciones de Mn (sangre de cordón, sangre o suero materno) también dificulta la comparación entre estudios (ver **Tabla 4 [artículo I]** y **tabla 6**).

Respecto a las concentraciones de As total en orina, así como de las diferentes especies, en el **Artículo II** observamos que estos los niveles fueron mucho más bajos que en áreas donde los niveles de iAs en el agua son altos (>10 µg/L) (Chou et al., 2014; Christian et al., 2006; Gardner et al., 2011; Laine et al., 2015; Wang et al., 2021; Zhang et al., 2021). En comparación, los niveles de As en agua en España durante los años de estudio (2004-2008) obtuvieron una media de 0,4 µg/L, presentando concentraciones mayores a 10 µg/L entre un 0,06 y 0,10% de las determinaciones (Palau Miguel et al., 2008, 2010). No obstante, las concentraciones urinarias de MMA, iAs, y AB observadas en el presente estudio fueron más altas que los observados en otras zonas donde los niveles de As en agua de consumo son bajos, como EEUU, Croacia o Canadá

(Chen et al., 2021; Howe et al., 2020; Stajnko et al., 2019; Vaughan Watson et al., 2020) (ver **Tabla 4 [Artículo 2]** y **Tabla 7**).

Estudio	País	N	Mn matriz	Periodo	Edad gestacional	Concentraciones Mn (µg/L)	Estudios que evalúan relación exposición Mn prenatal y desarrollo neuropsicológico infantil (edad evaluación; evaluación neurodesarrollo; resultados principales)
Presente estudio (Artículo I)	Spain	1179	Suero materno	2003-2005	1T	1,45 (1.30-1.67)[1]	1 año; Desarrollo mental y psicomotor (BSDI) Ns para ninguna de las escalas de BSDI
Oppenheimer, (2022)	EEUU	373	Sangre de cordón	1993-1998	Al nacimiento	4,2 (1,6)[4]	Adolescentes; Memoria de trabajo* — para Memoria de trabajo verbal, —para Índice de memoria de trabajo
McRae (2022)	Mexico	633	Sangre materna	2007-2011	2T 3T	2T: 3,7 (11,1-17,0)[1] 3T: 8,3 (14,8-22,4)[1]	---
Miyashita (2021)	Japan	89273	Sangre materna	2011-2014	2T y 3T	15,3 (12,6-18,6) ng/g[1]	---
Lee (2021)	South Korea	502	Sangre materna	2008-2010	2T	12,6 (10,2-15,6)[1]	6 años; Coeficiente intellectual** Ns Mn prenatal y CI a los 6 años de edad.
Skogheim (2021)	Noruega	1034 controles 705 TDHA 397 TEA	Sangre materna	1999-2008	Alrededor 17 sg	Control: 9,91 (8,04-13,30)[1] TDHA :9,63 (7,60-12, 0)[1] TEA: 10,2 (8,33-13,5)[1]	Nacidos entre 2002 y 2009; Diagnósticos de TDHA y TEA Mayor riesgo de TEA en hijos de madre con niveles de Mn prenatal en el 4 cuartil, en comparación con el primer cuartil.Se observe un ligero patrón en forma de U en niños con diagnóstico de TDHA.
Shih (2021)	EEUU	125	Sangre maternal	2009-2010	6-32 sg	10,51 (4,79-20,72)[2]	---
Zhou (2021)	China	8169	Suero materno	2017-2018	1T	0,82 (0,75-0,89)[1]	---
Rivera-Núnez (2021)	Puerto Rico	948	Sangre materna	2011-2017	Alrededor de la 18 y 26 sg.	11,3 (9,0-13,9)[1]	---

Pesce (2021)	Francia	651	Sangre maternal Sangre cordón	2003-2006	24-28 sg Al nacimiento	Sangre materna: 10 (8-12)[1] Sangre cordón: 31 (24-41)[1]	---
Gong (2021)	China	196	Sangre materna	2019	Cualquier momento del embarazo	16,47 (9,60-25,54)[3]	---
Dai (2021)	China	1179	Sangre cordón	2009-2010	Al nacimiento	29,25 (6,84-316,73)[2]	---
Davies (2021)	Benin	262	Sangre materna	2014-2016	1T	11,2 (8,76-13,2)[1]	---
Kupsco (2020)	México	571	Sangre materna	2006	2T 3T Parto	2T: 14,5 (5,0)[4] 3T: 19,3 (6,80)[4] Parto: 25,1 (10,8)[4]	4-6 años; MSCA + en puntuaciones de memoria y cuantitativa, en niños cuyas madres presentaron niveles de ferritina normales. + en escala de memoria en ninos cuyas madres presentaron niveles

Tabla 6. Concentraciones de Mn en sangre o suero y medidos en mujeres embarazadas en otros estudios y actualización de estudios longitudinales que han evaluado la exposición prenatal a Mn (medido en sangre o Suero materno o de cordon) y el desarrollo neuropsicológico durante la infancia (actualización Tabla 5, Artículo 1).

Nota: Sg: semanas de gestación; N : tamaño muestral; T: trimestre de gestación; TEA: transtoro del espectro autista; TDAH: Trastorno por déficit de atención / hiperactividad, —: relación negativa; +: relación positiva.
[1]Mediana (p25 — p75); [2]Mediana (Mínimo — máximo); [3]Mediana (p5-p95); [4] Media (desviación estándar)
*Evaluado a través de Wide Range Assessment of Memory and Learning, segunda edición (WRAML2) Verbal Working Memory and Symbolic Working Memory subtests.

Estudio	País	Edad gestacional	N	Periodo	TAs	∑iAs	iAs	MMA	DMA	AB	%iAs	%MMA	%DMA
Presente estudio (artículo II)	España (GIP-VAL)	1T (13sg)	1017	2003-2008	35,6[5,8] (33,1-38,2)	7,7[5,8] (7,4-8,1)	0,33[5,8] (0,31-0,36)	0,34[5,8] (0,32-0,36)	6,8[5,8] (6,5-7,1)	20,17[5,8] (18,34-0,19)	4,3 [5,8] (4,0-4,5)	4,4[5,8] (4,2-4,6)	88,2[5,8] (87,6-88,7)
Ruan (2022)	China	1T 2T 3T	628	2013-2016	1T: 20,12[1,8] (14,61-28,0) 2T: 24,03[1,8] (18,02 -35,83) 3T: 24,73[1,8] (17,90 -35,24)	ND	ND	ND	ND	ND	ND	ND	ND
Wang (2021)	China	1T	1038	2014-2016	13,08[1, 9] (9,79-8,22)	ND	2,45[1, 9] (1,25-4,14)	1,04[1, 9] (0,62-1,65)	8,81[1, 9] (6,16-2,69)	ND	19,04[1, 9] (10,31-30,89)	7,78[1, 9] (5,03-11,04) [1, 9]	72,15[1, 9] (59,68-80,83)
Liu (2021)	Taiwan	3T	430	2000-2001	12,71 (0,98)[4,9]	ND	0,45[4,9] (0,026)	0,28[4,9] (0,017)	2,97[4,9] (0,13)	6,54[4,9] (0,51)	ND	ND	ND
Zhang (2021)	China	24-28 sg	411	2017-2018	ND	ND	As3+: 0,84[1,9] (0,59-1,24) As5+: 1,97[1,9] (1,60-2,48)	0,34[1,9] (0,26-0,54)	10,95[1,9] (7,54-16,43)[1,9]	6,29[1,9] (3,36-13,65)	ND	ND	ND
Chen*(2021)	EEUU	24-28sg	237	2009-2010	7,32 (6,55-8,18)[5,9]	4,46 (4,04-4,94)[5,9]	1,36[5,9] (1,02-1,82)	<LOD	3,93[5,9] (3,59-4,30)	<LOD	ND	ND	ND
Fano-Sizgorich (2021)	Perú	≤ 24 sg	147	2019	52,65[6,9] (33,31)	ND	5,27[6,9] (2,91)	ND	ND	ND	ND	ND	ND
Moradnia (2021)	Irán	1T	140	2019-2020	4,5[1, 8] (2,2-12,0)	ND	ND	ND	ND	ND	ND	ND	ND
Tsai (2021)	Taiwan	3T	370	2012-2015	53,72[7,8] (53,03)	29,14[7,8] (7,69)	ND	ND	ND	ND	ND	ND	ND
Rivera-Núñez (2021)	Puerto Rico	Todo el embarazo**	948	2011-2017	11,0[1,9] (6,6-8,3)	ND	ND	ND	ND	ND	ND	ND	ND

Tabla 7. Concentraciones urinarias de As y sus metabolitos medidos en mujeres embarazadas en otros estudios (actualización Tabla 4, **artículo II**)

Nota: Sg: semanas de gestación; T: trimestre de embarazo; N: tamaño muestral; ND: no disponible

[1] Mediana (p25 — p75); [2]Mediana (Mínimo — máximo); [3] Mediana (p5-p95); [4]Media (desviación estándar); [5]media geométrica (95%IC); [6] Media geométrica (desviación estándar geométrica); [7] mediana (rango intercuartil); [8] Concentraciones ajustadas por creatinina (µg/gramo de cretinina); [9]Concentraciones ajustadas por gravedad específica (µg/L).

*Estudio de casos- controles. Se muestran niveles únicamente de controles.**Tres medidas repetidas durante todo el embarazo.

6.1.2 Factores asociados a niveles As y Mn (artículo I y II)

En la presente tesis se han estudiado los factores sociodemográficos, ambientales, dietéticos y de estilos de vida que se asociaban con las concentraciones de Mn (artículo I) y a las diferentes especies del As (artículo II). En el caso del Mn en suero materno, la única variable dietética que se asoció fue el consumo de frutos secos, incluso tras el ajuste por otras variables dietéticas. Este tipo de alimento suele presentar niveles altos de Mn, llegando a concentraciones entre 12 y 25 mg/kg en diferentes estudios (Agency for Toxic Substances and Disease Registry, 2012; Filippini et al., 2018; Rose et al., 2010). Además, España es el tercer país de la UE en consumo per cápita de este producto, por detrás de Grecia e Italia (Food and Agriculture Organization of the United Nations, 2017). Esta asociación contrasta con otros estudios realizados en áreas donde los niveles de Mn en agua son bajos, como en el caso de España, en los cuales la mayor contribución a la exposición a Mn es a través del consumo de cereales (Filippini et al., 2018; Gimou et al., 2014; Rose et al., 2010; Rubio et al., 2009).

Respecto a los factores sociodemográficos, observamos que las mujeres que no trabajaron durante el primer trimestre de embarazo presentaron concentraciones de Mn más bajas que las trabajadoras. Este subgrupo de participantes presentó un consumo menor de frutos secos que las trabajadoras (5.1 vs. 7.1 g/día, $p < 0.01$), aunque el consumo de cereales fue más alto (107 vs. 96.6 g/día, $p < 0.01$).

En nuestro estudio (artículo II), el consumo de arroz fue uno de los factores que presentó una mayor relación con las concentraciones urinarias de As total y el resto de las especies de As, excepto con la AB. Se han identificado muy pocos estudios que hayan analizado los niveles de As (total y sus especies) en muestras de arroz en nuestro país (Marín et al., 2018; Otero et al., 2020; Signes-Pastor et al., 2016). En ellos se observa que, aunque este producto presenta los niveles más altos de TAs e iAs respecto a otros alimentos, la mayoría de las muestras de arroz analizadas presentaron niveles por debajo de los límites establecidos por la Comisión Europea (European Commission, 2015b). No obstante, el consumo de este tipo de producto en España es alto, siendo el segundo país de la UE con un mayor consumo, por detrás de Portugal (Food and Agriculture Organization of the United Nations, 2017).

La ingesta de pescado también se asoció de manera directa con las concentraciones urinarias de AB. Esta forma de As, considerada no tóxica, es la especie más abundante de As presente en el pescado (European Food Safety Authority, 2009). Igualmente, el consumo de este tipo de alimento se asoció positivamente, aunque en menor proporción, con las concentraciones de DMA. Además de la AB, en el pescado pueden detectarse otras formas complejas de As (arsenoazúcares y arsenolípidos), que se metabolizan a DMA tras la ingesta, (European Food

Safety Authority, 2009; Molin et al., 2012), lo que podría explicar esta asociación en nuestro estudio. En el año 2019, España fue el segundo país de la UE, por detrás de Portugal, con un mayor consumo per cápita de pescado y productos pesqueros, presentando un nivel de consumo de estos productos casi del doble que la media de la UE (European Market Observatory for Fisheries and Aquaculture Product, 2019). Finalmente, el consumo de otros tipos de alimentos también se relacionó con alguna de las especies de As; por ejemplo, la ingesta de legumbres se asoció de manera directa con las concentraciones de iAs, probablemente por ser un producto que presenta niveles relativamente altos de iAs (Agencia Catalana de Seguretat Alimentaria, 2017). Por otra parte, el consumo de carne roja se asoció con una disminución de las concentraciones de MMA en orina. En este alimento se encuentran otros elementos y nutrientes asociados con un mejor metabolismo del As, como el Zn o la vitamina B_{12}, lo que podría explicar esta relación inversa (Kurzius-Spencer et al., 2017).

Respecto a otros factores asociados a la exposición a As durante el embarazo, en nuestro estudio observamos que las madres participantes en la cohorte de Valencia presentaron concentraciones menores de TAs, AB y DMA, pero mayores de iAs y MMA, en comparación con las participantes de la cohorte de Gipuzkoa. El patrón dietético de las mujeres en cada área de estudio es diferente, presentando un mayor consumo de arroz, marisco y moluscos en el área de Valencia, lo que podría explicar esta variabilidad geográfica (ver **Tabla S4, artículo II**). De manera similar, en nuestro estudio observamos menores concentraciones de AB en mujeres nacidas en Latinoamérica, que podría explicarse por un menor consumo de pescado en comparación con las mujeres nacidas en España, lo que coincide con otros estudios (Caldwell et al., 2009).

6.1.3 Eficiencia en la metilación del As durante el embarazo (artículo II)

En nuestra población, las mujeres embarazadas presentaron una mayor eficiencia en la metilación de As, indicado por un mayor %DMA, y menores %MMA y %iAs, que la observada en otros estudios en los que también se evaluó dicha eficiencia durante el embarazo (ver **Tabla 4, artículo II** y **Tabla 8**). Como ya se ha comentado, actualmente el proceso de biotransformación del iAs no está completamente entendido. El esquema propuesto incluye diferentes procesos de reducción (de formas pentavalentes a trivalentes), y metilación (de iAs a MMA en un primer paso y a DMA en un segundo paso) (Cullen, 2014). En la mayor parte de la literatura se ha usado como forma para evaluar la eficiencia en la metilación del iAs las proporciones relativas de cada metabolito urinario (iAs, DMA y MMA) sobre la suma de estas tres especies (Agency for Toxic Substances and Disease Registry, 2007). No obstante, este método tiene diversas limitaciones: 1) este abordaje parece inapropiado en poblaciones con un alto consumo de pescado, debido a que se podría sobreestimar la exposición a iAs debido a las concentraciones de DMA procedentes del

metabolismo de las especies contenidas en el pescado (Navas-Acien et al., 2011); 2) los tres porcentajes (%DMA, %MMA y %iAs) están altamente correlacionados, lo que puede dificultar la interpretación de los resultados. Con el fin de minimizar estas dos limitaciones, en el presente estudio se ha evaluado la eficiencia en la metilación del As usando dos perspectivas diferentes: 1) calculando el porcentaje de las concentraciones calibradas de cada metabolito y 2) realizando un análisis de componentes principales (PCA) de los porcentajes de los metabolitos. En nuestro estudio, con el método de la calibración se modificaron los niveles de metabolitos, produciendo una disminución especialmente de las concentraciones de DMA, lo que se tradujo en un menor porcentaje relativo de este metabolito y, en consecuencia, a un mayor porcentaje de MMA e iAs. Con el método de PCA, los resultados del presente estudio coinciden con los trabajos anteriores (Balakrishnan et al., 2016; Gribble et al., 2015; Jansen et al., 2016; Spratlen et al., 2017). El primer componente, PC1, indica una correlación inversa entre el %iAs y el %DMA, que se ha interpretado como la capacidad de producir DMA, es decir, una mayor eficiencia en la metilación del iAs. El segundo componente, PC2, indica una correlación inversa entre el %MMA y el %iAs. Este segundo componente se ha interpretado como la capacidad de transformar iAs en MMA (primer paso del proceso del metabolismo del iAs). No obstante, debido a que no existe todavía un consenso sobre cuál es la mejor manera de evaluar la eficiencia en la metilación del As, la interpretación y comparación de los resultados es compleja y deben tomarse con cautela.

6.1.4 Factores asociados a la eficiencia en la metilación del As durante el embarazo (artículo II)

En nuestro estudio, las mujeres latinoamericanas presentaron una mayor eficiencia en la metilación del As respecto a las mujeres nacidas en España. Este resultado coincide con estudios previos, donde se ha observado que la eficiencia en la metilación se asocia con la etnia de los y las participantes (Balakrishnan et al., 2018; De Loma et al., 2019; Farzan et al., 2020; Hopenhayn-Rich et al., 1996). De hecho, en ciertas poblaciones de Sudamérica expuestas durante generaciones a altos niveles de As, se ha observado la presencia de variaciones genéticas en el gen *AS3MT* (*arsenito metiltransferasa*), siendo esta característica genética la que contribuye en mayor medida a la eficiencia en la metilación del As (Schlebusch et al., 2015; Vahter et al., 1995). No obstante, también podría ser posible que otras variables relacionadas con el origen de la madre pudieran estar confundiendo esta asociación (dieta, hábito tabáquico etc), por lo que es necesario seguir investigando esta relación.

Así mismo, un mayor IMC pregestacional se relacionó con una mayor eficiencia en la metilación. El mecanismo subyacente a esta relación, que también se ha observado en estudios previos (Bommarito et al., 2019; Shen et al., 2016), no está claro. Se ha postulado que diversos factores

relacionados con el IMC, como una peor función renal o la ingesta de ciertas proteínas, podrían asociarse con una mayor excreción de DMA (Duan et al., 2019; Peters et al., 2015; Vahter, 2007).

Otros factores asociados con la eficiencia en la metilación del As son la edad gestacional y el consumo de tabaco durante el primer trimestre del embarazo. Ambos factores se relacionan con el metabolismo de 1 carbono (OCM, por sus siglas en inglés), proceso esencial para la síntesis de S-adenosil metionina (SAM), principal donante del grupo metilo en la metilación del iAs (Abuawad et al., 2021). Por una parte, se ha sugerido que el OCM es más eficiente durante el periodo del embarazo, ya que se incrementa la síntesis de colina endógena, uno de los donantes del grupo metilo (Vahter, 2009). Por otra parte, el consumo de tabaco se ha asociado con una disminución en las concentraciones de nutrientes relacionados con el OCM, como la vitamina B_{12} o el folato (Mouhamed et al., 2011). Además, el Cd, un compuesto presente en el tabaco, parece unirse al GSH, uno de los antioxidantes involucrado en la reducción de las especies de As de pentavalentes a trivalentes. De hecho, en nuestra población, las concentraciones urinarias de Cd se asociaron directamente con un aumento del %MMA.

Finalmente, en nuestro estudio no encontramos ninguna asociación entre la ingesta estimada de nutrientes relacionados con el OCM (vitamina $B_{6,}$ vitamina B_{12} y folato) y la eficiencia en la metilación. Estos nutrientes son necesarios en la síntesis de SAM. Diversos estudios han encontrado asociaciones entre alguno de estos nutrientes y la metilación del As (Bozack et al., 2019; Gamble et al., 2005; Howe et al., 2017; Kurzius-Spencer et al., 2017). Sin embargo, los resultados son heterogéneos cuando esta relación se evalúa en mujeres embarazadas (Hall et al., 2007; Laine et al., 2018; Li et al., 2008). Como se ha comentado anteriormente, se ha observado que la metilación del As aumenta durante el curso del embarazo, por lo que la influencia de ciertos cofactores y donantes de grupos metilo pueda ser menor durante este periodo (Gardner et al., 2011). Por otra parte, es posible que el efecto de estos nutrientes sea más difícil de apreciar en poblaciones con suficiencia nutricional, como es el caso de nuestra población, y que coincide con el estudio de Howe et.al., (2014).

6.1.5 Asociación de la exposición prenatal a Mn y el desarrollo neuropsicológico evaluado durante la infancia (artículo I)

En este estudio de cohortes multicéntrico no se encontró ninguna asociación entre la exposición prenatal a Mn (evaluada a través de las concentraciones maternas de Mn en suero) y el desarrollo mental y psicomotor de los niños y niñas evaluado al año de edad. Hoy en día, la evidencia disponible sobre la exposición prenatal a Mn y el desarrollo neuropsicológico durante la infancia es escasa, y los resultados son todavía poco concluyentes. Dos revisiones sistemáticas recientes

han evaluado la relación entre la exposición a Mn durante edades tempranas, no solamente prenatal, y el desarrollo neuropsicológico infantil. La primera de ellas incluyó 37 estudios epidemiológicos, tanto transversales como longitudinales (Leonhard et al., 2019). Debido a la alta heterogeneidad en los biomarcadores y los instrumentos de evaluación del efecto utilizados, el tipo de diseño, y la diferencia entre las poblaciones analizadas (población general y comunidades altamente expuestas), los autores del estudio concluyeron que la evidencia hasta el momento no podía confirmar una asociación causal. En una revisión sistemática posterior se incluyeron, entre otros, diez estudios de cohortes que evaluaron la asociación entre los niveles prenatales a Mn y el desarrollo neuropsicológico en niños y niñas menores de 6 años. Los autores de la revisión concluyeron que una mayor exposición al Mn se asociaba negativamente con el neurodesarrollo infantil, especialmente con las habilidades cognitivas y motoras en edades tempranas (menores de 6 años) (Liu et al., 2020).

Como se ha comentado anteriormente, debido a la alta heterogeneidad entre los estudios, la comparabilidad de los resultados es compleja. En la Tabla 4 (artículo I) y Tabla 7 se presentan los estudios longitudinales sobre la exposición prenatal a Mn (medido en suero o sangre materna o sangre del cordón umbilical) y el desarrollo neuropsicológico en la infancia. En concordancia con nuestros resultados, diversos estudios no encontraron ninguna asociación estadísticamente significativa entre la exposición prenatal a Mn y el desarrollo neuropsicológico evaluado a los 6 años de edad en Corea del Sur (Lee et al., 2021), al año de edad en Costa Rica (Mora et al., 2018) y a los 6 meses y 3 y 6 años en Francia (Takser et al., 2003). Respecto a este último artículo, esta ausencia de asociación solo se observó cuando se utilizó como biomarcador de exposición el Mn medido en sangre materna. Sin embargo, los autores sí que encontraron una relación inversa entre las concentraciones de Mn medido en sangre de cordón y varias escalas del MSCA. En el estudio de Oppenheimer et al., (2022), llevado a cabo en EEUU, también se encontró una asociación negativa entre los niveles de Mn medidos en sangre de cordón y la escala de memoria de trabajo evaluada durante la adolescencia. Al contrario, en el estudio de Claus-Henn et al., (2017) la asociación inversa significativa se observó cuando la exposición fue el Mn medido en sangre materna, pero no en sangre de cordón. Otros estudios, sin embargo, han encontrado una asociación beneficiosa entre los niveles prenatales de Mn y el neurodesarrollo infantil (Irizar et al., 2021; Mora et al., 2015). Precisamente, en el estudio de Irizar et al., (2021) se evaluó la relación entre la exposición prenatal a Mn en suero y el desarrollo neuropsicológico evaluado a los 4-5 años de edad en mujeres y niños y niñas de las cohortes INMA Valencia e INMA Gipuzkoa, las mismas que en el presente trabajo. En esta investigación se encontró una asociación beneficiosa de las concentraciones maternas de Mn en suero durante el primer trimestre de embarazo (media 1,56 µg/L) y las escalas verbal, numérica y general del MSCA, pero únicamente en niñas.

En algunas de las investigaciones previas, la asociación observada entre la exposición prenatal a Mn y el neurodesarrollo infantil siguió un patrón de U-invertida, lo que puede sugerir que existe un umbral de niveles de Mn por encima del cual se evidencian efectos negativos en el neurodesarrollo (Chung et al., 2015; Lin et al., 2013; Muñoz-Rocha et al., 2018). No obstante, en nuestro estudio el uso de modelos no lineales no mejoró el ajuste de los modelos, lo que está en consonancia con el estudio de Mora et al., (2018). Las discrepancias entre nuestro trabajo y las investigaciones previas pueden ser debidas a que las concentraciones de Mn en nuestra población se mantengan dentro de unos límites donde este compuesto se comporta como un elemento esencial, sin producir efectos tóxicos (Agency for Toxic Substances and Disease Registry, 2012).

Finalmente, en el presente estudio se evaluó si la asociación pudiera ser modificada por ciertas variables. En primer lugar, el Mn y el Fe comparten mecanismos de absorción y transporte, y estudios experimentales han sugerido que estos dos elementos compiten en la barrera hematoencefálica (Agency for Toxic Substances and Disease Registry, 2012). En nuestro estudio, las mujeres que presentaron niveles de ferritina sérica por debajo de 15 µg/L mostraron niveles ligeramente mayores de Mn que las que presentaban niveles normales de ferritina, aunque estas diferencias no fueron estadísticamente significativas. Al incluir el término de interacción entre la ferritina y el Mn en los modelos que evaluaron la asociación entre las concentraciones maternas de Mn y las puntuaciones en las subescalas del test de Bayley, este tampoco fue significativo para ninguna de las dos escalas. Solo se han encontrado dos estudios, ambos llevados a cabo en México, que han evaluado esta relación y en ambos se observan diferencias en el efecto producido por la exposición de Mn prenatal sobre en neurodesarrollo infantil, dependiendo del estado férrico de la madre (Gunier et al., 2015; Kupsco et al., 2020). En el estudio de Gunier et al., (2015) (mediana de Mn evaluada en dentina prenatal: 0,51 AUC 55Mn:43Ca) se observó una asociación negativa entre los niveles prenatales de Mn y las escalas tanto mental como psicomotora, en niñas cuyas madres presentaron niveles bajos de hemoglobina (<11,6 g/dL). En el segundo estudio, se observó que los niños y niñas cuyas madres presentaban valores normales de ferritina (≥15 µg/L) y de hemoglobina (≥ 11g/dL) presentaron mejores puntuaciones en diversas escalas del MSCA evaluado a los 4-6 años de edad al aumentar las concentraciones de Mn prenatal (medias de Mn en sangre en segundo y tercer trimestre del embarazo: 14,5 y 19,3 µg/L, respectivamente) (Kupsco et al., 2020).

En segundo lugar, se ha evaluado una posible modificación de efecto debida al sexo de los participantes al año de edad. Diversos estudios han encontrado una modificación del efecto por esta variable, observando efectos adversos solo en niñas (Baeuer et al., 2021; Bauer et al., 2017; Broberg et al., 2019; Gunier et al., 2015). No obstante, en nuestro estudio no encontramos que

el sexo influyera en la asociación, lo que está en consonancia con otros estudios (Bouchard et al., 2007; Oulhote et al., 2014).

6.1.6 Efectos de la exposición prenatal a As y sus diferentes especies y efectos en el neurodesarrollo durante la infancia (artículo III)

En el presente trabajo, se ha descrito una relación negativa y estadísticamente significativa entre las concentraciones urinarias de MMA durante el primer trimestre del embarazo y las puntuaciones en las siguientes subescalas del MSCA a los 4-5 años de edad: general, numérica, memoria, memoria de trabajo, y función ejecutiva. Diversos estudios han evaluado la relación entre la exposición prenatal a iAs y el desarrollo neuropsicológico durante la infancia. En la cohorte de Bangladesh, un incremento en las concentraciones de ∑As durante el primer trimestre del embarazo se asociaron a peores puntuaciones en la escala de cociente intelectual verbal a los 5 años de edad, aunque únicamente en niñas (Hamadani et al., 2011). En la misma cohorte, el efecto negativo se observó en las escalas de comprensión verbal, velocidad de procesamiento, razonamiento de procesamiento, y la puntuación de desarrollo completo a los 10 años de edad, siendo el efecto en estas dos últimas escalas únicamente significativo en niñas (Vahter et al., 2020). Un estudio realizado en población nepalí encontró una asociación inversa entre las concentraciones de As medidas en sangre de cordón y la escala de regulación del estado de la escala neonatal de Brazelton, pero no se observó ninguna asociación con el neurodesarrollo a los 6 y 24 meses de edad en la misma población (Parajuli et al., 2013, 2014; Parajuli et al., 2015a). En una cohorte de nacimiento llevada a cabo en China, las concentraciones de As en suero de cordón se asociaron con un mayor riesgo de retraso en el desarrollo en el ámbito social a los 6 meses de edad, aunque, igual que en Bangladesh, esta asociación únicamente se observó en niñas (Liang et al., 2020). Finalmente, en un estudio llevado a cabo en una zona de alta exposición a As y otros tóxicos (área de minería de oro) en Tanzania no se observó ninguna asociación entre las concentraciones urinarias de As durante el segundo trimestre del embarazo y el desarrollo neuropsicológico de los niños y niñas entre 6 y 12 años de edad (Nyanza et al., 2021).

Como se observa, la evidencia respecto a la exposición prenatal a As y su efecto en el desarrollo neuropsicológico durante la infancia es todavía insuficiente para establecer una conclusión definitiva. Además, existen diferencias sustanciales entre los estudios que dificultan la comparación. Por una parte, las concentraciones de As han sido analizadas en diferentes matrices (sangre y suero de cordón u orina materna en diferentes momentos del embarazo). Por otra parte, el biomarcador de exposición también varió entre los diferentes estudios, siendo el más utilizado el As total o la suma de formas orgánicas e inorgánicas (DMA, MMA e iAs), pero ninguno de ellos ha evaluado el efecto de cada especie de As individualmente. De hecho, hasta donde

sabemos, el trabajo incluido en esta tesis (Artículo III) es el primer estudio epidemiológico que ha evaluado la asociación entre las diferentes especies prenatales de As y el desarrollo neuropsicológico en la infancia en poblaciones expuestas a niveles bajos de As.

En nuestro estudio, las concentraciones prenatales de MMA se asociaron con una peor puntuación en varias subescalas del MSCA a los 4-5 años de edad, aunque la magnitud del efecto fue pequeña. Como se ha comentado, no hemos encontrado estudios que hayan evaluado las concentraciones prenatales de MMA y el neurodesarrollo infantil. Únicamente se han localizado dos estudios transversales que evaluaron la asociación entre las concentraciones postnatales de MMA y la función cognitiva en niños, encontrando que mayores concentraciones de MMA se relacionan con peores resultados en el neurodesarrollo infantil (Hsieh et al., 2014; Rosado et al., 2007). Sin embargo, la comparación con estos estudios debe hacerse con precaución debido a las diferencias entre las concentraciones y el tipo de diseño de los estudios.

Además, en el presente estudio se encontró una relación positiva entre las concentraciones de TAs y AB y la puntuación en la escala verbal. Este resultado podría explicarse por el consumo de pescado. Este alimento es la mayor fuente de AB, la cual es el mayor contribuidor del As total (European Food Safety Authority, 2009). Además de ser fuente de diversos tóxicos, el consumo de pescado provee de nutrientes esenciales relacionados con un mejor neurodesarrollo, como proteínas y ácidos grasos poliinsaturados de cadena larga (FAO/WHO, 2010; Julvez et al., 2016). Aunque los modelos finales se ajustaron por el consumo de pescado, es posible que el cuestionario de frecuencia alimentaria no recogiera totalmente la variabilidad en la ingesta materna de pescado y hubiera cierta confusión residual.

6.1.7 Eficiencia en la metilación del arsénico durante el embarazo y asociación con el neurodesarrollo durante la infancia (artículo 3)

El metabolismo del As está considerado como un mecanismo de detoxificación. No obstante, las formas intermedias producidas durante este proceso, especialmente las formas trivalentes, podrían tener una alta reactividad y toxicidad (Nesnow et al., 2002; Styblo et al., 2000). De hecho, el aumento en el %MMA ha estado relacionado con un incremento de efectos negativos en salud, como aumento del riesgo de padecer cáncer de pulmón, de piel y de mama, o enfermedades cardiovasculares (Abuawad et al., 2021; Gamboa-Loira et al., 2017; Kuo et al., 2017). En nuestro estudio, hemos observado una asociación inversa entre el %MMA y las puntuaciones en la escala de memoria. Únicamente hemos encontrado un estudio caso-control en el que se analizó la asociación entre el metabolismo del As (evaluado mediante %DMA, %MMA y %iAs) y el riesgo de padecer retraso en el neurodesarrollo a los 5-6 años de edad en niños y niñas de Taiwan. En este

estudio los participantes con porcentajes de MMA urinario mayores a 2,14 presentaron un riesgo mayor de padecer retraso en el neurodesarrollo (Odds Ratio [IC95%]: 2,35 [1,22 a 4,53]) (Hsueh et al., 2016).

En el presente trabajo se ha evaluado la posible modificación del efecto producido por diferentes nutrientes y elementos, como por ejemplo las vitaminas B_6 y B_{12}, el folato, el Zn, el Se y el estado férrico. Así, se ha observado que los niños y niñas cuyas madres presentaron mayores %iAs y deficiencia de hierro (ferritina <15 µg / L) obtuvieron puntuaciones más bajas en las escalas general, verbal y de memoria. De la misma manera, los niños y niñas cuyas madres presentaron niveles más bajos de Zn urinario (<363.7 µg/L) presentaron peores puntuaciones en las tres escalas motoras, conforme aumentaba el %MMA. Solo unos pocos estudios han evaluado la relación entre las concentraciones de Fe y/o Zn y el metabolismo del As, obteniendo resultados heterogéneos (Kordas et al., 2016, 2017; Li et al., 2008). En estudios experimentales se ha observado que mayores concentraciones de Fe disminuyen la bioaccesibilidad del As en el intestino, aumentando su metabolismo, lo que puede llevar a una diminución en su toxicidad (Clemente et al., 2019; Liu et al., 2013; Yu et al., 2016). Por su parte, en modelos animales, la administración de Zn parece restaurar los niveles de glutatión, disminuidos por la exposición al As, e induce la producción de metalotioneína, lo que podría proteger contra la toxicidad producida por el As (Ganger et al., 2016; Kumar et al., 2010).

6.1.8 Interacción entre la eficiencia en la metilación del As y Mn y efectos en el neurodesarrollo durante la infancia (artículo III)

En el presente estudio hemos descrito una interacción entre la eficiencia en la metilación del As y los niveles de Mn en suero materno. Los niños y niños cuyas madres presentaron una menor eficiencia en la metilación de As (denotado por mayor %iAs) y concentraciones más altas de Mn (≥1,44 µg/L) obtuvieron puntuaciones mayores en la escala motora global.

Como se ha comentado anteriormente, el Mn es un metal esencial que participa en numerosos procesos metabólicos, como la síntesis y el metabolismo de neurotransmisores (Agency for Toxic Substances and Disease Registry, 2012; Santamaria y Sulsky, 2010). Algunos estudios epidemiológicos han evaluado la interacción entre las concentraciones de As y Mn, pero no se han localizado trabajos que hayan estudiado la posible interacción con el metabolismo del As. En un estudio transversal realizado en los EE. UU, los niños y niñas que presentaron concentraciones de As y Mn en cabello por encima de la mediana (14,1 ppm para As y 471,5 ppm para Mn) obtuvieron puntuaciones más bajas en la escala completa y en la escala del cociente de inteligencia verbal (Wright et al., 2006). En un estudio longitudinal en Bangladesh, la exposición

prenatal a As potenció los efectos neurotóxicos de los niveles prenatales de Mn a los 24 meses de edad (Valeri et al., 2017). Finalmente, en otro estudio previo del proyecto INMA, no se encontró ninguna interacción entre los niveles de Mn y As placentarios detectables sobre el desarrollo neuropsicológico a los 4-5 años de edad (Freire et al., 2018). Los resultados de los estudios experimentales son también contradictorios. En el estudio de Andreade et al., (2017) se encontró que la exposición a una mezcla de Pb, Mn y As aumentaba la toxicidad de la actividad motora en ratas, debido a una disminución en la actividad de la acetilcolinesterasa cerebral. Por el contrario, en Biswas et al. (2019) se observó una relación antagonista entre las concentraciones de Mn y As y su asociación con el aprendizaje y la memoria espacial en ratones(Biswas et al., 2019). Los autores de esta investigación sugirieron que el Mn podría prevenir la acumulación de As en el cerebro. Sin embargo, la evidencia actual no permite establecer cuál es la relación entre estos dos compuestos, por lo que se necesitan más estudios que evalúen la modificación del efecto producida por el Mn en la relación entre la exposición a As y el impacto en el desarrollo neuropsicológico durante la infancia.

6.1.9 Posibles mecanismos de neurotoxicidad por exposición a Mn y As

Se han descrito diferentes mecanismos involucrados en los efectos neurotóxicos del Mn. Por una parte, el Mn se acumula en la mitocondria de neuronas y astrocitos de ciertas zonas del cerebro, particularmente en el ganglio basal (específicamente en el *globus pallidus*). La acumulación de Mn puede producir inflamación, disfunción mitocondrial, estrés oxidativo y apoptosis de células dopaminérgicas, provocando una alteración en la neurotransmisión dopaminérgica, lo que puede llevar a una afectación del comportamiento que incluye déficits cognitivos (por ejemplo, en la función ejecutiva) y de la función motora (Balachandran et al., 2020; Miah et al., 2020; Pajarillo et al., 2021).

Por otra parte, la exposición de Mn parece provocar una desregulación en las enzimas relacionadas con la síntesis y metabolismo del glutatión (GSH). Una disminución de la forma reducida de este tripéptido, con gran poder antioxidante, provocaría la producción excesiva de especies reactivas de oxígeno (ROS), induciendo estrés oxidativo, inflamación y apoptosis especialmente en el ganglio basal (Lucchini, Aschner, Kim, et al., 2015; Miah et al., 2020; Nyarko-Danquah et al., 2020; Pajarillo et al., 2021).

Además, se ha observado que el estrés oxidativo causado por la exposición a Mn podría provocar cambios en la funcionalidad de la enzima ADN metiltransferasa, encargada de unir los grupos metilo con el ADN, induciendo así cambios en la metilación del ADN (Ijomone et al., 2020). Estos

cambios epigenéticos podrían afectar la expresión de genes cruciales para el neurodesarrollo (Salinas et al., 2020)

Igualmente, otro mecanismo de neurotoxicidad propuesto es la alteración de los sistemas de neurotransmisores y su actividad en el cerebro. Un ejemplo es el efecto del Mn en el sistema colinérgico del cerebro. El Mn tiene la capacidad de unirse al transportador de la colina reduciendo así su transferencia a través de la barrera hematoencefálica. El déficit de colina, un componente clave necesario para la síntesis del neurotransmisor acetilcolina, podría contribuir potencialmente a efectos adversos en funciones cognitivas como el aprendizaje y la memoria (Balachandran et al., 2020; Pajarillo et al., 2021; Peres et al., 2016).

Por su parte, los mecanismos de neurotoxicidad del As y sus especies no están todavía claros. Uno de los mecanismos propuestos es el estrés oxidativo. La metilación del As requiere del consumo de grandes cantidades de GSH, provocando una disminución de este antioxidante, lo que lleva a la generación de ROS. Estudios experimentales han observado que la exposición prenatal a As provoca una disminución de GSH y un aumento en las concentraciones de ROS, que se acumulan en ciertas áreas del sistema nervioso central, específicamente en la región de la corteza central, el hipocampo y el cuerpo estriado (Chandravanshi et al., 2018; Luo y Shu, 2015; Masjosthusmann et al., 2021; Mishra y Flora, 2008). La corteza prefrontal es una región relacionada con la memoria, la percepción y otros procesos cognitivos (Siddiqui et al., 2008). Este estrés oxidativo podría afectar a la función mitocondrial, e inducir peroxidación lipídica y apoptosis en las células neuronales (Mochizuki, 2019). Concretamente, se ha descrito que la función mitocondrial es especialmente importante en la neurogénesis del cerebro en desarrollo, por lo que una perturbación en este sistema puede desencadenar efectos adversos en el desarrollo de procesos cognitivos (Masjosthusmann et al., 2021).

Por otra parte, recientemente se ha descrito la intervención de mecanismos epigenéticos en la aparición de efectos neurotóxicos debidos a una exposición temprana a iAs (Bailey y Fry, 2014). Concretamente, tanto la metilación de ADN como la metilación del iAs comparten el mismo donante de grupos metilo, la SAM, por tanto, el metabolismo del iAs podría dar lugar a la hipometilación del ADN y los consecuentes cambios en la expresión génica. Sin embargo, estos cambios no están totalmente claros en la actualidad, ya que estudios experimentales han observado hipo e hipermetilación del ADN tras la exposición prenatal a As, dependiendo del sexo (Luo y Shu, 2015; Masjosthusmann et al., 2021).

Aunque la evidencia todavía es muy escasa, otros mecanismos que podrían intervenir en la neurotoxicidad del iAs son la desregulación de ciertos sistemas de neurotransmisores y la disrupción endocrina. Se ha observado que la exposición a iAs provoca alteraciones en los

sistemas dopaminérgico y colinérgico en ratas, ambos con importantes roles en la actividad motora, el aprendizaje y la memoria (Chandravanshi et al., 2019; Chandravanshi et al., 2014). Por otro lado, la exposición a este tóxico se ha asociado con una disrupción en el eje hipotálamo-hipófisis-suprarrenal, incrementando la producción de corticosteroides, relacionados con respuestas neuroendocrinas y conductuales. También se ha encontrado evidencia de que el As es un disruptor endocrino tiroideo, aunque la evidencia disponible es inconsistente (Sun et al., 2016).

6.2 LIMITACIONES Y FORTALEZAS

6.2.1 Limitaciones del estudio

El presente estudio presenta una serie de limitaciones que es necesario considerar:

- Una limitación a destacar es la pérdida en el seguimiento propia de los estudios de cohortes. Esta pérdida en el seguimiento podría representar un sesgo en la estimación de ciertas asociaciones exposición-resultado (sesgo de selección). En el Proyecto INMA se han realizado, y se realizan, esfuerzos para minimizar las pérdidas de participantes, como por ejemplo, el envío de boletines periódicos con resultados y consejos de salud, la página web dirigida a las familias con diferente información sobre el proyecto, y el contacto con las familias para informar de resultados en las evaluaciones que requieran asistencia o seguimiento clínico, entre otros. Estas acciones han dado como resultado una baja tasa de pérdidas a lo largo del tiempo de seguimiento que comprenden los trabajos presentados (Guxens et al., 2012). Sin embargo, se observa una mayor participación de familias con un perfil socioeconómico más privilegiado, lo que podría afectar a la representatividad del estudio. En todo caso, en los estudios que componen esta tesis se han realizado diferentes análisis para explorar cualquier diferencia entre la población incluida y no incluida, y se ha tenido en cuenta esta información en la interpretación de los resultados.

- Otra de las limitaciones del estudio ha sido la evaluación de las exposiciones (Mn y As prenatal) en una medición única durante el embarazo. Por una parte, esta evaluación puntual a través de los biomarcadores elegidos (Mn sérico y As urinario) podría reflejar únicamente exposiciones a corto plazo. Por otra parte, se ha sugerido que las concentraciones de ambos compuestos varían a lo largo del embarazo, incrementándose los niveles de Mn, que también se ha descrito para la eficiencia del metabolismo del As hacia el final de este periodo (Gao et al., 2019; Gardner et al., 2011; Spencer, 1999; Takser et al., 2004).

- Junto a lo anterior, este estudio carece de evaluación de la exposición postnatal de ambos compuestos. La vulnerabilidad del sistema nervioso central se extiende desde el inicio del embarazo hasta la adolescencia. Esto quiere decir que tanto la exposición durante el periodo prenatal como el postnatal a Mn y As podría afectar el desarrollo neuropsicológico durante la infancia. No obstante, gracias a los seguimientos realizados a los participantes del proyecto

INMA, es posible que en futuras investigaciones se pueda tener en cuenta la exposición postnatal a estos compuestos a lo largo de la infancia.

- Algunas covariables y variables confusoras presentaron datos perdidos, lo que ha podido llevar a una pérdida de potencia estadística cuando estas variables se incluyeron en los modelos finales.

- En el artículo III se realizaron múltiples análisis, especialmente cuando se evaluó la modificación de efecto incluyendo el término de interacción en los modelos. Esto podría llevar a posibles asociaciones significativas por azar. Sin embargo, estos análisis se refieren a diferentes compuestos con características particulares y diferentes variables resultado que describen dimensiones específicas del neurodesarrollo, por lo que se consideran independientes. No obstante, en el artículo únicamente se discutieron los resultados que obtuvieron un p valor <0,01.

- Aunque en este estudio se han analizado como covariables y factores de confusión múltiples variables sociodemográficas, clínicas, dietéticas y de estilos de vida, así como otros compuestos, existe falta de información sobre otras variables que podrían influir en el desarrollo cognitivo (como el entorno familiar de crecimiento postnatal) y la relación entre la metilación del As, la exposición a As y a Mn. Esto hace que no sea posible descartar la posibilidad de confusión residual en las relaciones entre las exposiciones y los resultados del desarrollo neuropsicológico.

- De manera similar, aunque en los trabajos sobre As se ha evaluado la influencia de ciertos nutrientes relacionados con el metabolismo de un carbono (vitaminas B_6 y B_{12}, folato), otros no han podido ser analizados, como la colina, metionina y betaína. Es posible que en el futuro se puedan realizar estimaciones de estos nutrientes gracias a la información dietética disponible, o mediciones directas de las concentraciones de los mismos.

- Finalmente, en la presente tesis tampoco se ha evaluado la influencia de factores genéticos en el metabolismo del As, ni tampoco en la relación entre las exposiciones y el neurodesarrollo infantil. Existe evidencia que los factores genéticos tienen un efecto sobre los mecanismos toxicocinéticos y de neurotoxicidad de ambos compuestos (Broberg et al., 2015) Actualmente y relacionado con la estancia realizada en la Universidad de Gotemburgo, se está llevando a cabo un estudio para evaluar la influencia de ciertos polimorfismos genéticos en el metabolismo del arsénico y su posible neurotoxicidad en los niños y niñas participantes en la cohorte.

6.2.2 Fortalezas del estudio

Por otro lado, este estudio cuenta con fortalezas importantes:

- El diseño prospectivo de la cohorte INMA ha permitido realizar un seguimiento longitudinal durante el embarazo y la primera infancia, lo que supone diversas ventajas. Por una parte, este diseño ha permitido evaluar las exposiciones de manera previa a los posibles efectos, siendo esta relación temporal uno de los criterios para establecer una relación causa-efecto. Además, se ha podido obtener una amplia variedad de información sobre las características de los participantes, estilos de vida, variables dietéticas y niveles de otros metales y elementos esenciales, que pueden actuar como confusores o modificadores de efecto en la relación entre las exposiciones y el desarrollo neuropsicológico.

- Otra de las ventajas de este estudio es que se ha evaluado la exposición en las primeras etapas del embarazo, lo que permite evaluar el papel de exposiciones durante etapas tempranas del desarrollo. Además, la evaluación del desarrollo neuropsicológico a lo largo de la infancia y hasta la adolescencia, permitirá identificar efectos a largo plazo o persistencia de los mismos.

- Además, se debe destacar el gran tamaño muestral de los estudios incluidos en la tesis. Esto ha permitido aumentar la potencia estadística de los análisis.

- Otra de las fortalezas de la presente investigación es su carácter multicéntrico. La inclusión de participantes de dos áreas con características sociodemográficas, ambientales y de estilos de vida heterogéneas permite que los resultados puedan ser más generalizables a la población general.

- La determinación de las especies del As ha permitido evaluar los factores asociados a cada uno de los metabolitos del As de manera específica. Esto permite dar una idea de las fuentes de exposición de cada especie de As de manera individual en nuestra población. Igualmente, la especiación del As también ha permitido evaluar el efecto de las concentraciones de cada metabolito en concreto. De hecho, hasta donde sabemos, en esta tesis se incluye el primer estudio que analiza el efecto individual de la exposición prenatal a cada especie de As en el desarrollo neuropsicológico en la infancia.

- En este estudio, además de la exposición a las diferentes especies de As, se ha estudiado el proceso intermedio de metilación de este compuesto, así como su relación con el desarrollo neuropsicológico en la infancia. Además, la eficiencia en la metilación del As se ha evaluado desde diferentes aproximaciones, lo que permite la comparabilidad con estudios previos. De

la misma manera que en el punto anterior, hasta donde hemos encontrado, el artículo II de la presente tesis es el primer estudio que ha evaluado la relación entre la eficiencia en la metilación materna durante el embarazo y el desarrollo neuropsicológico en la infancia.

- Finalmente, debido a las características propias de nuestra población, en la que se observa un consumo alto de pescado, se ha usado el método de calibración de los diferentes metabolitos del As propuesto por Jones et al., (2016) con el propósito de minimizar la influencia de las especies orgánicas de As presentes en el pescado, y consideradas no tóxicas. No obstante, se ha realizado un análisis de sensibilidad usando como variables de exposición los porcentajes de los metabolitos sin calibrar, así como ajustando el modelo por el consumo de pescado.

6.3 IMPLICACIONES EN SALUD PÚBLICA Y POLÍTICAS SANITARIAS

Como se ha comentado anteriormente, la etapa prenatal supone un periodo de alta vulnerabilidad a la exposición a sustancias tóxicas o a niveles inadecuados de elementos esenciales. Los cambios que se producen durante este periodo, así como en el período postnatal temprano, pueden llevar a una mayor susceptibilidad a padecer efectos adversos durante la infancia y la edad adulta (Grandjean et al., 2019). Por ello, esta etapa supone una oportunidad especialmente importante para poner en marcha intervenciones de protección, prevención y promoción de la salud (Britto et al., 2017). No obstante, es necesario seguir acumulando evidencia sobre los posibles efectos en salud debido a estas exposiciones, lo que permitirá el establecimiento de niveles de referencia y recomendaciones contextualizadas a cada región, incluidas zonas con baja exposición a estos compuestos.

Los resultados derivados de la presente tesis aportan evidencia sobre los efectos en el desarrollo neuropsicológico infantil asociados a la exposición a niveles concretos de Mn y de las distintas especies de As. Aunque las concentraciones de Mn en suero materno en nuestro estudio no se asociaron con el neurodesarrollo al año de edad, sí que hemos encontrado que las concentraciones urinarias de MMA tuvieron un efecto negativo en diversas escalas del desarrollo neuropsicológico a los 4-5 años. Estos resultados indican que se debe seguir trabajando en la monitorización del As, así como sus diferentes especies, y en el desarrollo de acciones que permitan prevenir y/o limitar su exposición durante esta etapa vulnerable (recomendaciones dietéticas, control de alimentos y agua, control de las industrias, entre otras). Igualmente, se debe continuar analizando los efectos de estas exposiciones prenatales sobre el neurodesarrollo infantil durante la infancia y la adolescencia, para evaluar la persistencia de los efectos negativos o la posible aparición de efectos adversos retardados en edades más avanzadas.

Respecto a los niveles, nuestra población de estudio presentó concentraciones de As total más bajas que las observadas en zonas con altos niveles de iAs en agua, sin embargo, las de MMA y iAs fueron más altas que en otras zonas con niveles a iAs en agua similares a la nuestra. Este resultado muestra que la exposición a iAs, así como a otras especies intermedias, a través de la dieta debería ser evaluada, especialmente en etapas vulnerables, como durante el embarazo y la infancia. En el año 2021, la Autoridad Europea de Seguridad Alimentaria (EFSA) publicó un

informe donde se describía la exposición alimentaria crónica a iAs en población europea, estimando que la exposición alimentaria a este compuesto en mujeres embarazadas variaba de media de 0,04 a 0,14 µg/kg de peso al día (European Food Safety Authority et al., 2021). A día de hoy no se ha establecido un nivel de referencia de ingesta tolerable. En el 2009, la EFSA propuso un rango de valores del límite inferior de confianza de la dosis de referencia (BMDL01) entre 0,3 y 8 µg/kg de peso corporal/día (European Food Safety Authority, 2009). No obstante, en el mismo informe se estimó que la exposición en consumidores promedio y altos se sitúa muy cerca o incluso dentro del rango de la BMDL01, por lo que no se puede descartar riesgo para la salud (European Food Safety Authority, 2009; European Food Safety Authority et al., 2021). Respecto al Mn, la EFSA propuso en el año 2013 una ingesta adecuada de Mn de 3 mg al día en población general y mujeres embarazadas y lactantes (EFSA Panel on Dietetic Products Nutrition and Allergies, 2013), aunque debido a la falta de evidencia, este organismo no pudo establecer una ingesta superior tolerable. Por su parte, el Institute of Medicine estableció una ingesta diaria tolerable no superior a 11 mg, aunque esta recomendación no se ha revisado en más de quince años (Institute of Medicine, 2006). La acumulación de evidencia sobre efectos en salud es necesaria para la reevaluación de estas recomendaciones.

Finalmente, la evaluación de factores relacionados con la exposición a Mn y cada una de las especies de As, así como los asociados a la eficiencia en la metilación del As posibilita identificar grupos vulnerables a la exposición. Esta información podría servir para establecer acciones de salud pública dirigidas a estos subgrupos. Además, este análisis también permite identificar cuáles son los alimentos que más contribuyen a la exposición de estos compuestos en nuestra población. Esta información puede servir de base para la evaluación de los niveles de estos compuestos en productos alimentarios específicos, así como establecer políticas encaminadas a la reducción de la exposición y la protección de la salud de la población general.

6.4 FUTURAS INVESTIGACIONES

La evaluación de la exposición a contaminantes y otros elementos esenciales durante periodos vulnerables y sus efectos sobre la salud infantil es un tema complejo que cuenta aún con muchas cuestiones sin resolver. La generación de información y evidencia ayudará a realizar intervenciones específicas de prevención, protección y promoción de la salud, así como desarrollar legislaciones que disminuyan la exposición a tóxicos durante etapas vulnerables, y a lo largo de la vida, generando ambientes más saludables para la población.

En este sentido, el Proyecto INMA dispone de información longitudinal y de alta calidad que permite generar conocimiento sobre la exposición a estos compuestos, tanto en la etapa prenatal como postnatal. Durante cerca de veinte años se han realizado seguimientos periódicos a las familias participantes en el proyecto, por lo que se podría evaluar los niveles de Mn y de As, así como sus especies, desde la infancia temprana hasta la adolescencia, estudiando distintos factores, dietéticos, sociodemográficos y otros, relacionados con dichas exposiciones. Además, a lo largo del seguimiento, en diferentes momentos de la infancia, se han recogido muestras biológicas (entre ellas, orina a los 4, 7, 9 y 15 años), lo que permitirá evaluar la eficiencia en la metilación del As en la etapa postnatal de manera longitudinal, obteniendo información sobre los factores que se asocian a este proceso de biotransformación.

Igualmente, debido al carácter longitudinal del proyecto INMA y gracias a la participación de las familias de las que se dispone información desde los primeros estadíos del embarazo, es posible estudiar cuál es el impacto de las exposiciones tempranas a estos compuestos a lo largo de la vida. Las cohortes de nacimiento suponen una de las mejores oportunidades para poder estudiar la relación entre el ambiente durante etapas tempranas del desarrollo y los efectos en salud a largo plazo, tal y como postula la hipótesis de los orígenes de la salud y enfermedad en el desarrollo (DOHaD). En este sentido, además de posibles efectos en salud relacionados con la exposición temprana a As y Mn, también se podrán explorar diversos mecanismos de acción, como cambios epigenéticos (longitud de telómero, metilación del ADN), marcadores de estrés oxidativo e inflamación, así como la permanencia de los posibles cambios.

Por otra parte, existe evidencia de que los factores genéticos juegan un papel importante en la toxicocinética y neurotoxicidad de ambos compuestos, y, en consecuencia, en la vulnerabilidad a la exposición (Broberg et al., 2015). Siguiendo esta línea de investigación, actualmente está en marcha la investigación de la influencia de ciertos polimorfismos genéticos en la eficiencia en la

metilación del As durante el embarazo. Por otro lado, también se pretende evaluar la influencia de la genética en la asociación entre la exposición prenatal al As (total y sus diferentes metabolitos) y el desarrollo neuropsicológico infantil. En un futuro se podría investigar la influencia de la genética sobre el metabolismo del Mn y su asociación con el desarrollo neuropsicológico infantil.

La exposición a estos compuestos podría tener otros efectos sobre la salud infantil. Durante las visitas de seguimiento se han evaluado diferentes aspectos relacionados con la salud a lo largo de la infancia y la adolescencia (antropometría, desarrollo sexual, presión arterial, salud respiratoria etc). Esto permitirá ampliar la evaluación de los posibles efectos en la salud asociados a la exposición prenatal a Mn y As, durante estos periodos. De hecho, siguiendo esta línea, ya se están desarrollando diversas investigaciones que tratan de obtener evidencia sobre la relación de la exposición prenatal a As y efectos en la salud respiratoria infantil.

Finalmente, cabe destacar que los mecanismos tóxicos de estos compuestos son complejos (formas de relación no lineales, exposiciones múltiples e interacción entre compuestos, efectos retardados, etc) y no están totalmente entendidos en la actualidad. Además, los efectos en salud dependen también de la interacción entre distintos elementos, tanto tóxicos como esenciales. Como hemos observado en los resultados de la presente tesis, parece que el Mn modifica la relación entre el metabolismo del As y el neurodesarrollo. Todo esto hace necesario seguir estudiando la intrincada relación entre distintos compuestos con la salud y el desarrollo a lo largo de la vida. En el proyecto INMA se han evaluado numerosos metales tóxicos y esenciales en diferentes biomarcadores y momentos (embarazo, varios momentos durante la infancia, y se prevé analizarlos también en la adolescencia). Esto permitirá seguir estudiando y aportando información sobre la interacción entre distintos compuestos y sus efectos en salud.

CAPÍTULO VII. CONCLUSIONES

CONCLUSIONES

Relacionado con la exposición prenatal a Mn, As y sus metabolitos y factores asociados a la exposición:

- Las concentraciones de Mn en suero durante el primer trimestre del embarazo en nuestra población de estudio fueron más bajas que las observadas en otros estudios.

- El factor dietético que se asoció a las concentraciones prenatales de Mn en suero fue el consumo de frutos secos.

- Las madres trabajadoras presentaron menores concentraciones de Mn en suero.

- Las concentraciones de As total en orina, así como sus diferentes especies durante el primer trimestre del embarazo, en nuestra población fueron considerablemente menores que las observadas en áreas con niveles altos de As en el ambiente. No obstante, las concentraciones de MMA, iAs y AB fueron mayores que las observadas en áreas con bajo nivel de exposición a este compuesto.

- En relación con los factores asociados a la exposición a As, el consumo de arroz durante el embarazo se asoció con un aumento en las concentraciones urinarias de As total, ∑As, DMA, MMA e iAs. Por otra parte, el consumo de pescado se asoció positivamente con las concentraciones de todos los metabolitos evaluados exceptuando el MMA y el iAs. El consumo de carne roja, legumbres y vegetales también se relacionó con las concentraciones urinarias de diferentes metabolitos de As durante el primer trimestre del embarazo.

- Las mujeres pertenecientes a la cohorte de Valencia presentaron concentraciones urinarias de iAs y MMA mayores que las mujeres de la cohorte de Gipuzkoa. Las mujeres que habían nacido en Latinoamérica presentaron concentraciones más bajas de AB. Por su parte, pertenecer a una clase social más baja se asoció con mayores niveles de MMA en orina. Finalmente, el IMC se relacionó de manera inversa con las concentraciones de MMA e iAs.

Relacionado con la eficiencia en la metilación del As durante el embarazo y los factores asociados a dicha eficiencia:

- Las mujeres embarazadas de nuestro estudio presentaron una mayor eficiencia en la metilación de As, indicado por un mayor %DMA, y menores %MMA y %iAs, que los observados en otros estudios que evaluaron dicha eficiencia durante el embarazo.

- Las mujeres pertenecientes a la cohorte de Gipuzkoa, nacidas en Latinoamérica, con mayor IMC pregestacional y no fumadoras presentaron una mayor eficiencia en la metilación del As durante el primer trimestre del embarazo. Así mismo, la edad gestacional se relacionó inversamente con el %MMA.

- En nuestro estudio no encontramos ninguna asociación entre la eficiencia de metilación de As y la ingesta estimada de nutrientes relacionados con el metabolismo de 1 carbono. No obstante, la ingesta estimada de hierro y zinc, así como las concentraciones urinarias de cadmio se relacionaron con una menor eficiencia en la metilación del As.

Relacionado con la exposición prenatal a Mn, As y sus metabolitos y el desarrollo neuropsicológico durante la infancia:

- En el presente trabajo no se encontró ninguna asociación entre la exposición prenatal a Mn, evaluada a través de las concentraciones maternas de Mn en suero durante el primer trimestre del embarazo, y el desarrollo mental y psicomotor de los niños y niñas al año de edad. Tampoco se observó una relación no lineal ni modificación del efecto debido al estado férrico de la madre o al sexo del niño.

- Las concentraciones urinarias de MMA evaluadas en el primer trimestre del embarazo se relacionaron de manera negativa con las subescalas general, numérica, de memoria, de memoria de trabajo, y de función ejecutiva de la prueba McCarthy de aptitudes y psicomotricidad para niños y niñas, evaluado a los 4-5 años de edad. Sin embargo, la magnitud del efecto observada fue pequeña.

- También se ha observado una relación positiva entre las concentraciones prenatales de TAs y AB con la subescala verbal de la prueba McCarthy de aptitudes y psicomotricidad para niños y niñas, evaluado a los 4-5 años de edad.

Relacionado con el metabolismo del As y el desarrollo neuropsicológico durante la infancia:

- Se ha encontrado evidencia de una relación inversa entre el %MMA prenatal y la función de la memoria a los 4-5 años de edad. Por otra parte, un mayor %iAs se relacionó positivamente con la subescala de motricidad gruesa.

- La asociación entre la eficiencia en la metilación del As y el desarrollo neuropsicológico de los niños y niñas a los 4-5 años parece estar influenciada por las concentraciones maternas de algunos nutrientes y elementos. Así, los niños y niñas cuyas madres presentaron niveles más bajos de Zn en orina y ferritina sérica durante el embarazo obtuvieron peores puntuaciones con una eficiencia en la metilación del As decreciente.

Relacionado con la interacción entre la eficiencia en la metilación del As y Mn y efectos en el neurodesarrollo durante la infancia:

- En el presente trabajo se ha observado una interacción entre la eficiencia en la metilación del As y los niveles de Mn en suero materno. Los niños y niñas cuyas madres presentaron una menor eficiencia en la metilación de As (denotado por mayor %iAs) y concentraciones más altas de Mn (≥1,44 µg / L) obtuvieron puntuaciones mayores en las escalas de rendimiento perceptivo, motricidad gruesa y motora fina.

- La evidencia de la relación entre la exposición prenatal a estos compuestos y el efecto tóxico sobre el desarrollo neuropsicológico en la infancia es todavía no concluyente. Aunque nuestros resultados sugieren un efecto tóxico de las concentraciones prenatales de MMA sobre el desarrollo neuropsicológico, la complejidad de la propia exposición y el metabolismo de este compuesto, así como la interacción con otros elementos y variables, hace que estos resultados deban tomarse con cautela.

- Se necesitan más estudios epidemiológicos en poblaciones expuestas a bajos niveles ambientales de As y Mn, como la del presente trabajo, para mejorar el conocimiento sobre el impacto en salud de la exposición a estos compuestos durante periodos críticos, como en el desarrollo prenatal y la infancia. Además, futuros trabajos también ayudarán a aumentar la evidencia sobre los factores que pueden afectar al metabolismo de ambos elementos. En combinación con estudios epidemiológicos bien diseñados, existe la necesidad de seguir investigando sobre el mecanismo de la neurotoxicidad tanto del As como del Mn. En conjunto, este conocimiento podría utilizarse para proponer nuevas recomendaciones y estrategias de salud pública.

CONCLUSIONS

With regard to prenatal exposure to Mn, As and its metabolites and factors associated with exposure:

- Serum Mn concentrations during the first trimester of pregnancy were lower in our study population than those observed in other studies.
- The dietary factors that were associated with prenatal serum Mn concentrations were consumption of nuts.
- Working mothers had lower serum Mn concentrations.
- In our population the concentrations of total As in urine, as well as its different species during the first trimester of pregnancy, were considerably lower than those observed in areas with high levels of As in the environment. However, MMA, iAs and AB concentrations were higher than those observed in areas with low As exposure.
- Concerning factors associated with As exposure, rice consumption during pregnancy was associated with increased urinary concentrations of total As, ∑As, DMA, MMA and iAs. Similarly, fish consumption was positively associated with concentrations of all metabolites assessed except MMA and iAs. Consumption of red meat, legumes and vegetables was also associated with urinary concentrations of different As metabolites during the first trimester of pregnancy.
- The women in the Valencia cohort had higher urinary concentrations of iAs and MMA than those in the Gipuzkoa cohort. Women born in Latin America had lower AB concentrations. In addition, belonging to a lower social class was associated with higher urinary MMA levels. Finally, BMI was inversely related to MMA and iAs concentrations.

With regard to As methylation efficiency during pregnancy and the factors associated with this efficiency:

- Pregnant women in our study had a higher As methylation efficiency, indicated by higher %DMA, and lower %MMA and %iAs than those observed in other studies assessing As methylation efficiency during pregnancy.

- Women belonging to the Gipuzkoa cohort, born in Latin America, with a higher pregestational BMI and non-smokers had a higher As methylation efficiency during the first trimester of pregnancy. Likewise, gestational age was inversely related to %MMA.
- In our study we found no association between As methylation efficiency and estimated intake of nutrients related to 1-carbon metabolism. However, estimated iron and zinc intakes as well as urinary cadmium concentrations were associated with lower As methylation efficiency.

With regard to prenatal exposure to Mn, As and its metabolites and neuropsychological development during childhood:

- In the present study, no association was found between prenatal Mn exposure, as assessed by maternal serum Mn concentrations during the first trimester of pregnancy, and the children's mental and psychomotor development at one year of age. There was also non- lineal relation and effect modification due to maternal iron status or the child's sex.
- Urinary MMA concentrations assessed in the first trimester of pregnancy were negatively related to the general, numerical, memory, working memory, and executive function subscales of the McCarthy test of children's abilities and psychomotor skills, assessed at 4-5 years of age, although the magnitude of the observed effect was small.
- A positive relationship was also observed between prenatal TAs and AB concentrations and the verbal subscale of the McCarthy test of children's abilities and psychomotor skills, assessed at 4-5 years of age.

With regard to As metabolism and neuropsychological development during childhood:

- Evidence has been found of an inverse relationship between prenatal %MMA and memory function at 4-5 years of age. Moreover, a higher %iAs was positively related to the gross motor subscale.

- The association between As methylation efficiency and children's neuropsychological development at 4-5 years of age seems to be influenced by maternal concentrations of certain nutrients and elements. Thus, children whose mothers had lower levels of urinary Zn and serum ferritin during pregnancy scored worse with a decreasing As methylation efficiency.

With regard to the interaction between As methylation efficiency and Mn and effects on neurodevelopment during childhood:

- In the present study, an interaction between As methylation efficiency and maternal serum Mn levels was observed. Children whose mothers had a lower As methylation efficiency (denoted by higher %iAs and %MMA) and lower Mn concentrations presented lower scores on perceptual, gross motor and fine motor performance scales.
- The evidence of the relationship between prenatal exposure to these compounds and the toxic effect on neuropsychological development in childhood is still inconclusive. Although our results suggest a toxic effect of prenatal MMA concentrations on neuropsychological development, the complexity of the exposure itself and the metabolism of this compound, as well as the interaction with other elements and variables, mean that these results should be taken with caution.

- Further epidemiological studies in populations exposed to low environmental levels of As and Mn, like the one used in the present study, are needed to improve knowledge on the health impact of exposure to these compounds during critical periods, such as prenatal development and childhood. In addition, future work will also help to increase the evidence on the factors that may affect the metabolism of the two elements. In combination with well-designed epidemiological studies, there is a need for further research on the mechanism of neurotoxicity of both As and Mn. Taken together, this knowledge could be used to propose new public health recommendations and strategies.

REFERENCIAS BIBLIOGRÁFICAS

Abbassi-Ghanavati, M., Greer, L. G. y Cunningham, G. (2009). Pregnancy and Laboratory Studies A Reference Table for Clinicians. *Obstetrics & Gynecology*, *114*(6), 1326-1331.

Abbott, L. C. y Nigussie, F. (2021). Mercury Toxicity and Neurogenesis in the Mammalian Brain. *International Journal of Molecular Sciences Review*, *22*, 7520. https://doi.org/10.3390/ijms22147520

Abdul-Hussein, A., Kareem, A., Tewari, S., Bergeron, J., Briollais, L., Challis, J. R., Davidge, S. T., Delrieux, C., Fortier, I., Goldowitz, D., Nepomnaschy, P., Wazana, A. y Connor, K. L. (2020). Early life risk and resiliency factors and their influences on developmental outcomes and disease pathways: A rapid evidence review of systematic reviews and meta-analyses. *Journal of Developmental Origins of Health and Disease*. https://doi.org/10.1017/S2040174420000689

Abuawad, A., Bozack, A. K., Saxena, R. y Gamble, M. V. (2021). Nutrition, one-carbon metabolism and arsenic methylation. *Toxicology*, *457*, 152803. https://doi.org/10.1016/j.tox.2021.152803

Agence nationale de sécurité sanitaire de l'alimentation de l'environnement et du travail. (2011). Second French Total Diet Study. En *ANSES Opinion* (Número June).

Agencia Catalana de Seguretat Alimentaria. (2017). *Contaminantes químicos. Estudio de dieta total en Cataluña, 2012*. Generalitat de Catalunya. Departament de Salut.

Agència Catalana de Seguretat Alimentaria. (2020). *Contaminants químics . V Estudi de dieta total a Catalunya Metalls pesants , dioxines (PCDD / F) i bifenils policlorats (PCB)*. Generalitat de Catalunya. Departament de Salut.

Agency for Toxic Substances and Disease Registry. (2007). *Toxicological profile for arsenic*. Division of Toxicology and Environmental Medicine. https://doi.org/10.1088/0004-6256/139/6/2557

Agency for Toxic Substances and Disease Registry. (2012). *Toxicological profile for Manganese* (Número September). Agency for Toxic Substances and Disease Registry.

Agency for Toxic Substances and Disease Registry. (2016). *Addendum to the toxicological profile for arsenic* (Número February). Division of Toxicology and Human Health Sciences.

Aggett, P., Nordberg, G. F. y Nordberg, M. (2015). Essential Metals: Assessing Risks from Deficiency and Toxicity. En G. Nordberg, B. Fowler, & M. Nordberg (Eds.), *Handbook on the Toxicology of Metals* (Fourth Edi, Vol. 1, pp. 281-297). Elsevier. https://doi.org/10.1016/B978-0-444-59453-2.00014-7

Alao, M. E., Perin, J., Brooks, W. A., Hossain, L., Goswami, D., Zaman, K., Yunus, M., Khan, M. A., Jahan, Y., Ahmed, D., Slavkovich, V., Graziano, J., Prosperi, C., Higdon, M., Deloria-Knoll, M., O' Brien, K. L. y George, C. M. (2021). Urinary arsenic is associated with wasting and underweight status in young children in rural Bangladesh. *Environmental Research*, *195*, 110025. https://doi.org/10.1016/j.envres.2020.110025

Almeida, D. L., Pavanello, A., Saavedra, L. P., Pereira, T. S., de Castro-prado, M. A. A. y Cezar, P. (2019). Environmental monitoring and the developmental origins of health and disease. *Journal of Developmental Origins of Health and Disease*, *10*, 608-615.

Andreade, V., Mateus, M. L., Batoreu, M. C., Aschner, M. y dos Santos, A. M. (2017). Toxic Mechanisms Underlying Motor Activity Changes Induced by a Mixture of Lead, Arsenic and Manganese. *EC Pharmacology and toxicity*, *3*(2), 31-42.

Ashley-Martin, J., Dodds, L., Arbuckle, T. E., Ettinger, A. S., Shapiro, G. D., Fisher, M., Monnier, P., Morisset, A. S., Fraser, W. D. y Bouchard, M. F. (2018). Maternal and cord blood manganese (Mn) levels and birth weight: The MIREC birth cohort study. *International Journal of Hygiene and Environmental Health*, *221*(6), 876-882. https://doi.org/10.1016/j.ijheh.2018.05.015

Baeuer, J. A., White, R. F., Coull, B. A., Austin, C., Oppini, M., Zoni, S., Fedrighi, C., Cagna, G., Placidi, D., Guazzetti, S., Yang, Q., Bellinger, D. C., Webster, T. F., Wright, R. O., Smith, D., Horton, M., Lucchini, R. G., Arora, M. y Claus Henn, B. (2021). Critical windows of susceptibility in the association between manganese and neurocognition in Italian adolescents living near ferro-manganese industry. *Neurotoxicology*, *87*, 51-61. https://doi.org/10.1016/j.neuro.2021.08.014

Bailey, K. A. y Fry, R. C. (2014). Arsenic-Associated Changes to the Epigenome: What Are the Functional Consequences? *Current environmental health reports*, *1*(1), 22-34. https://doi.org/10.1007/s40572-013-0002-8

Balachandran, R. C., Mukhopadhyay, S., McBride, D., Veevers, J., Harrison, F. E., Aschner, M., Haynes, E. N. y Bowman, A. B. (2020). Brain manganese and the balance between essential roles and neurotoxicity. *Journal of Biological Chemistry*, *295*(19), 6312-6329. https://doi.org/10.1074/jbc.REV119.009453

Balakrishnan, P., Jones, M. R., Vaidya, D., Tellez-Plaza, M., Post, W. S., Kaufman, J. D., Bielinski, S. J., Taylor, K., Francesconi, K., Goessler, W. y Navas-Acien, A. (2018). Ethnic, geographic, and genetic differences in arsenic metabolism at low arsenic exposure: A preliminary analysis in the multi-ethnic study of atherosclerosis (MESA). *International Journal of Environmental Research and Public Health*, *15*, 1179. https://doi.org/10.3390/ijerph15061179

Balakrishnan, P., Vaidya, D., Franceschini, N., Voruganti, V. S., Gribble, M. O., Haack, K., Laston, S., Umans, J. G., Francesconi, K. A., Goessler, W., North, K. E., Lee, E., Yracheta, J., Best, L. G., MacCluer, J. W., Kent, J., Cole, S. A. y Navas-Acien, A. (2016). Association of Cardiometabolic Genes with Arsenic Metabolism Biomarkers in American Indian Communities: The Strong Heart Family Study (SHFS). *Environmental Health Perspectives*, *125*, 15-22. https://doi.org/10.1289/EHP251

Barker, D. J. (2007). The origins of the developmental origins theory. *Journal of Internal Medicine*, *261*(5), 412-417. https://doi.org/10.1111/j.1365-2796.2007.01809.x

Barouki, R., Melén, E., Herceg, Z., Beckers, J., Chen, J., Karagas, M., Puga, A., Xia, Y., Chadwick, L., Yan, W., Audouze, K., Slama, R., J, H., Grandjean, P., Kawamoto, T. y Nohara, K. (2018). Epigenetics as a mechanism linking developmental exposures to long-term toxicity. *Environment International*, *114*, 77-86. https://doi.org/:10.1016/j.envint.2018.02.014.

Barr, D. B., Bishop, A. y Needham, L. L. (2007). Concentrations of xenobiotic chemicals in the maternal-fetal unit. *Reproductive Toxicology*, *23*(3), 260-266. https://doi.org/10.1016/j.reprotox.2007.03.003

Bauer, J. A., Claus Henn, B., Austin, C., Zoni, S., Fedrighi, C., Cagna, G., Placidi, D., White, R. F., Yang, Q., Coull, B. A., Smith, D., Lucchini, R. G., Wright, R. O. y Arora, M. (2017). Manganese in teeth and neurobehavior: Sex-specific windows of susceptibility. *Environment International*, *108*, 299-308. https://doi.org/10.1016/j.envint.2017.08.013

Bayley, N. (1977). *Escalas Bayley de Desarrollo Infantil.* TEA Ediciones.

Bellinger, D. C. (2012). A strategy for comparing the contributions of environmental chemicals and other risk factors to neurodevelopment of children. *Environmental Health Perspectives*, *120*(4), 501-507. https://doi.org/10.1289/ehp.1104170

Biswas, S., Anjum, A., Banna, H. U., Rahman, M., Siddique, A. E., Karim, Y., Nikkon, F., Haque, A., Hossain, K. y Saud, Z. A. (2019). Manganese attenuates the effects of arsenic on neurobehavioral and biochemical changes in mice co-exposed to arsenic and manganese. *Environmental Science and Pollution Research*, *26*(28), 29257-29266. https://doi.org/10.1007/s11356-019-06112-y

Bjorklund, G., Chartrand, M. S. y Aaseth, J. (2017). Manganese exposure and neurotoxic effects in children. *Environmental Research*, *155*(February), 380-384. https://doi.org/10.1016/j.envres.2017.03.003

Boffetta, P. y Borron, C. (2019). Low-Level Exposure to Arsenic in Drinking Water and Risk of Lung and Bladder Cancer: A Systematic Review and Dose–Response Meta-Analysis. *Dose-Response: An International journal*, *17*(3), 1-9. https://doi.org/10.1177/1559325819863634

Bommarito, P. A., Xu, X., González-Horta, C., Sánchez-Ramirez, B., Ballinas-Casarrubias, L., Luna, R. S., Pérez, S. R., Hernández Ávila, J. E., García-Vargas, G. G., Del Razo, L. M., Stýblo, M., Mendez, M. A. y Fry, R. C. (2019). One-carbon metabolism nutrient intake and the association between body mass index and urinary arsenic metabolites in adults in the Chihuahua cohort. *Environment International*, *123*, 292-300. https://doi.org/10.1016/j.envint.2018.12.004

Bouchard, M., Laforest, F., Vandelac, L., Bellinger, D. y Mergler, D. (2007). Hair manganese and hyperactive behaviors: Pilot study of school-age children exposed through tap water. *Environmental Health Perspectives*, *115*(1), 122-127. https://doi.org/10.1289/ehp.9504

Bozack, A. K., Hall, M. N., Liu, X., Ilievski, V., Lomax-luu, A. M., Parvez, F., Siddique, A. B., Shahriar, H., Uddin, M. N., Islam, T., Graziano, J. H. y Gamble, M. V. (2019). Folic acid supplementation enhances arsenic methylation : results from a folic acid and creatine supplementation randomized controlled trial in Bangladesh. *The American journal of clinical nutrition*, *109*, 380-391. https://doi.org/10.1093/ajcn/nqy148

Britto, P. R., Lye, S. J., Proulx, K., Yousafzai, A. K., Matthews, S. G., Vaivada, T., Perez-Escamilla, R., Rao, N., Ip, P., Fernald, L. C. H., MacMillan, H., Hanson, M., Wachs, T. D., Yao, H., Yoshikawa, H., Cerezo, A., Leckman, J. F. y Bhutta, Z. A. (2017). Nurturing care: promoting early childhood development. *The Lancet*, *389*(7), 91-102. https://doi.org/10.1016/S0140-6736(16)31390-3

Broberg, K., Engström, K. y Ameer, S. (2015). Gene-Environment Interactions for Metals. En G. Nordberg, B. Fowler, & M. Nordberg (Eds.), *Handbook on the Toxicology of Metals*. Elsevier.

Broberg, K., Taj, T., Guazzetti, S., Peli, M., Cagna, G., Pineda, D., Placidi, D., Wright, R. O., Smith, D. R., Lucchini, R. G. y Wahlberg, K. (2019). Manganese transporter genetics and sex modify the association between environmental manganese exposure and neurobehavioral outcomes in children. *Environment International*, *130*, 104908. https://doi.org/10.1016/j.envint.2019.104908

Calderón, J., Navarro, M. E., Jimenez-Capdeville, M. E., Santos-Diaz, M. del S., Golden, A., Rodriguez-Leyva, I., Borja-Aburto, V. y Díaz-Barriga, F. (2001). Exposure to arsenic and lead and neuropsychological development in Mexican children. *Environmental Research*, *85*(2), 69-76. https://doi.org/10.1006/enrs.2000.4106

Caldwell, K. L., Jones, R. L., Verdon, C. P., Jarrett, J. M., Caudill, S. P. y Osterloh, J. D. (2009). Levels of urinary total and speciated arsenic in the US population: National Health and Nutrition Examination Survey 2003-2004. *Journal of Exposure Science and Environmental Epidemiology*, *19*(1), 59-68. https://doi.org/10.1038/jes.2008.32

Campbell, R. C. J., Stephens, W. E. y Meharg, A. A. (2014). Consistency of arsenic speciation in global tobacco products with implications for health and regulation. *Tobacco Induced Diseases*, *12*(24), 1-8. https://doi.org/10.1186/s12971-014-0024-5

Challenger, F. (1945). Biological Methylation. *Chemical reviews*, *36*(3), 315-361. https://doi.org/10.1021/cr60115a003

Chandravanshi, L., Gupta, R. y Shukla, R. K. (2019). Arsenic-Induced Neurotoxicity by Dysfunctioning Cholinergic and Dopaminergic System in Brain of Developing Rats. *Biological Trace Element Research*, *189*, 118-133. https://doi.org/https://doi.org/10.1007/s12011-018-1452-5

Chandravanshi, L. P., Gupta, R. y Shukla, R. K. (2018). Developmental Neurotoxicity of Arsenic: Involvement of Oxidative Stress and Mitochondrial Functions. *Biological Trace Element Research*, *186*(1), 185-198. https://doi.org/10.1007/s12011-018-1286-1

Chandravanshi, L. P., Shukla, R. K., Sultana, S., Pant, A. B. y Khanna, V. K. (2014). Early life arsenic exposure and brain dopaminergic alterations in rats. *International Journal of Developmental Neuroscience*, *38*, 91-104. https://doi.org/10.1016/j.ijdevneu.2014.08.009

Chang, J. Y., Park, J. S., Shin, S., Yang, H. R., Moon, J. S. y Ko, J. S. (2015). Mercury Exposure in Healthy Korean Weaning-Age Infants: Association with Growth, Feeding and Fish Intake. *International journal of environmental research and public health*, *12*(11), 14669-14689. https://doi.org/10.3390/ijerph121114669

Chen, P., Bonhorst, J. y Aschner, M. (2018). Manganese metabolism in humans. *Frontiers in Bioscience*, *23*(October), 1655-1679.

Chen, W. J., Davis, E. M., Stoner, J. A., Robledo, C., Goodman, J. R., Garwe, T., Janitz, A. E., Xu, C., Hwang, J. y Peck, J. D. (2021). Urinary total arsenic and arsenic methylation capacity in pregnancy and gestational diabetes mellitus: A case-control study. *Chemosphere*, *271*, 129828. https://doi.org/10.1016/j.chemosphere.2021.129828

Chen, Y., Han, Y. H., Cao, Y., Zhu, Y. G., Rathinasabapathi, B. y Ma, L. Q. (2017). Arsenic transport in rice and biological solutions to reduce arsenic risk from rice. *Frontiers in Plant Science*, *8*(268). https://doi.org/10.3389/fpls.2017.00268

Chen, Y., Wu, F., Liu, M., Parvez, F., Slavkovich, V., Eunus, M., Ahmed, A., Argos, M., Islam, T., Rakibuz-Zaman, M., Hasan, R., Sarwar, G., Levy, D., Graziano, J. y Ahsan, H. (2013). A prospective study of arsenic exposure, arsenic methylation capacity, and risk of cardiovascular disease in Bangladesh. *Environmental Health Perspectives*, *121*(7), 832-838. https://doi.org/10.1289/ehp.1205797

Cheyns, K., Waegeneers, N., Van de Wiele, T. y Ruttens, A. (2017). Arsenic Release from Foodstuffs upon Food Preparation. *Journal of Agricultural and Food Chemistry*, *65*(11), 2443-2453. https://doi.org/10.1021/acs.jafc.6b05721

Chou, W. C., Chung, Y. T., Chen, H. Y., Wang, C. J., Ying, T. H., Chuang, C. Y., Tseng, Y. C. y Wang, S. L. (2014). Maternal arsenic exposure and DNA damage biomarkers, and the associations with birth outcomes in a general population from Taiwan. *PLoS ONE*, *9*(2), e86398. https://doi.org/10.1371/journal.pone.0086398

Christian, W. J., Hopenhayn, C., Centeno, J. A. y Todorov, T. (2006). Distribution of urinary selenium and arsenic among pregnant women exposed to arsenic in drinking water. *Environmental Research*, *100*(1), 115-122. https://doi.org/10.1016/j.envres.2005.03.009

Chung, S. E., Cheong, H. K., Ha, E. H., Kim, B. N., Ha, M., Kim, Y., Hong, Y. C., Park, H. y Oh, S. Y. (2015). Maternal blood manganese and early neurodevelopment: The mothers and children's environmental health (MOCEH) study. *Environmental Health Perspectives*, *123*(7), 717-722. https://doi.org/10.1289/ehp.1307865

Claus Henn, B., Bellinger, D. C., Hopkins, M. R., Coull, B. A., Ettinger, A. S., Jim, R., Hatley, E., Christiani, D. C. y Wright, R. O. (2017). Maternal and cord blood manganese concentrations and early childhood neurodevelopment among residents near a mining-impacted superfund site. *Environmental Health Perspectives*, *125*(6), 1-9. https://doi.org/10.1289/EHP925

Clemente, M. J., Cimbalo, A., Chiocchetti, G., Devesa, V. y Vélez, D. (2019). Dietary Compounds to Reduce in Vivo Inorganic Arsenic Bioavailability. *Journal of Agricultural and Food Chemistry*, *67*, 9032-9038. https://doi.org/10.1021/acs.jafc.9b03372

Cohen Hubal, E. A., de Wet, T., Du Toit, L., Firestone, M. P., Ruchirawat, M., van Engelen, J. y Vickers, C. (2014). Identifying important life stages for monitoring and assessing risks from exposures to environmental contaminants: Results of a World Health Organization review. *Regulatory Toxicology and Pharmacology*, *69*(1), 113-124. https://doi.org/10.1016/j.yrtph.2013.09.008

Council of the European Union. (2015). *Consolidated text: Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption*.

Cullen, W. R. (2014). Chemical mechanism of arsenic biomethylation. *Chemical Research in Toxicology*, *27*(4), 457-461. https://doi.org/10.1021/tx400441h

Dai, Y., Zhang, J., Qi, X., Wang, Z., Zheng, M., Liu, P., Jiang, S., Guo, J., Wu, C. y Zhou, Z. (2021). Cord blood manganese concentrations in relation to birth outcomes and childhood physical growth: A prospective birth cohort study. *Nutrients*, *13*(12). https://doi.org/10.3390/nu13124304

Davies, S., Briand, V., Accrombessi, M., Fievet, N., Le Bot, B., Durand, S., Agbota, G., Yovo, E., Vianou, B., Sossou, D., Martin-Prevel, Y., Massougbodji, A., Cot, M., Glorennec, P. y Bodeau-Livinec, F. (2021). Pre-conception serum ferritin concentrations are associated with metal concentrations in blood during pregnancy: A cohort study in Benin. *Environmental Research*, *202*(April). https://doi.org/10.1016/j.envres.2021.111629

De Loma, J., Tirado, N., Ascui, F., Levi, M., Vahter, M., Broberg, K. y Gardon, J. (2019). Elevated arsenic exposure and efficient arsenic metabolism in indigenous women around Lake Poopó Bolivia. *Science of the Total Environment*, *657*, 179-186. https://doi.org/10.1016/j.scitotenv.2018.11.473

Desai, G., Barg, G., Queirolo, E. I., Vahter, M., Peregalli, F., Mañay, N. y Kordas, K. (2018). A cross-sectional study of general cognitive abilities among Uruguayan school children with low-level arsenic exposure, potential effect modification by methylation capacity and dietary folate. *Environmental Research*, *164*, 124-131. https://doi.org/10.1016/j.envres.2018.02.021

Di Giovanni, P., Di Martino, G., Scampoli, P., Cedrone, F., Meo, F., Lucisano, G., Romano, F. y Staniscia, T. (2020). Arsenic exposure and risk of urothelial cancer: Systematic review and meta-analysis. *International Journal of Environmental Research and Public Health*, *17*, 3105. https://doi.org/10.3390/ijerph17093105

Duan, Y., Wang, X., Zhang, J., Ye, P., Cao, R., Yang, X., Bai, Y. y Wu, H. (2019). Body mass index is an independent predictive factor for kidney function evaluated by glomerular filtration rate in a community-dwelling population. *Eating and Weight Disorders*, *24*, 731-738. https://doi.org/10.1007/s40519-017-0434-5

EFSA. (2021). *Extensive literature search on organic arsenic in food*. https://www.efsa.europa.eu/en/call/extensive-literature-search-organic-arsenic-food

EFSA Panel on Dietetic Products Nutrition and Allergies. (2013). Scientific Opinion on Dietary Reference Values for manganese. *EFSA Journal*, *11*(11), 1-44. https://doi.org/10.2903/j.efsa.2013.3419

Estarlich, M., Ballester, F., Aguilera, I., Fernández-Somoano, A., Lertxundi, A., Llop, S., Freire, C., Tardón, A., Basterrechea, M., Sunyer, J. y Iñiguez, C. (2011). Residential Exposure to Outdoor Air Pollution during Pregnancy and Anthropometric Measures at Birth in a Multicenter Cohort in Spain. *Environmental health Perspectives*, *119*(9), 1333-1338.

Estarlich, M., Ballester, F., Davdand, P., Llop, S., Esplugues, A., Fernández-Somoano, A., Lertxundi, A., Guxens, M., Basterrechea, M., Tardón, A., Sunyer, J. y Iñiguez, C. (2016). Exposure to ambient air pollution during pregnancy and preterm birth: A Spanish multicenter birth cohort study. *Environmental Research*, *147*, 50-58. https://doi.org/10.1016/J.ENVRES.2016.01.037

Etzel, R. A. y Landrigan, P. J. (2014). Children´s Exquisite Vulnerability to Environmental Exposures. En P. J. Landrigan & R. A. Etzel (Eds.), *Textbook of children´s environmental health* (pp. 18-27). Oxford University Press.

Eum, J.-H., Cheong, H.-K., Ha, E. H., Ha, M., Kim, Y., Hong, Y. C., Park, H. y Chang, N. (2014). Maternal blood manganese level and birth weight: A MOCEH birth cohort study. *Environmental Health*, *13*(31), 1-7. https://doi.org/10.1186/1476-069X-13-31

European Chemical Agency. (2017). *Committee for Risk Assessment. Opinion on Arsenic acid and its inorganic salts*.

European Commission. (2004). *Directive 2004/107/EC of the European Parliament and of the Council of 15 December 2004 relating to arsenic, cadmium, mercury, nickel and polycyclic aromatic hydrocarbons in ambient air*.

European Commission. (2006). *Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006*.

European Commission. (2009). *Directive 2009/30/EC of the European Parliament and of the council of 23 April 2009 amending Directive 98/70/EC as regards the specification of petrol, diesel and gas-oil and introducing a mechanism to monitor and reduce greenhouse gas emissions and amend*. Official Journal of the European Union.

European Commission. (2015a). Commission delegated Regulation (EU) 2016/127 of 25 September 2015. *Official Journal of the European Union*, *4*, 1-25.

European Commission. (2015b). *Commission Regulation (EU) 2015/1006 of 25 June 2015 amending Regulation (EC) No 1881/2006 as regards maximum levels of inorganic arsenic in foodstuffs*.

European Commission. (2020a). Commission Implementing Regulation (EU) 2020/2087 of 14 December 2020. En *Official Journal of the European Union*. https://doi.org/10.2903/j.efsa.2019

European Commission. (2020b). *Directive (EU) 2020/2184 of 16 December 2020*. Official Journal of the European Union.

European Commission Employment Social Affairs and Inclusion. (2011). *Recommendation from the Scientific Committee on Occupational Exposure Limits for manganese and inorganic manganese compounds* (Número December).

European Environment Agency. (2020). Air quality in Europe — 2020 report - EEA Report No 9/2020. En *Publications Office of the European Union* (Número 09).

European Food Safety Authority. (2009). Scientific Opinion on Arsenic in Food. *European Food Safety Authority Journal*, *7*(10). https://doi.org/10.2903/j.efsa.2009.1351.

European Food Safety Authority. (2014). Dietary exposure to inorganic arsenic in the European population. *European Food Safety Authority Journal*, *12*(3). https://doi.org/10.2903/j.efsa.2014.3597

European Food Safety Authority, Arcella, D., Cascio, C. y Gómez Ruiz, J. Á. (2021). Chronic dietary exposure to inorganic arsenic. *EFSA Journal*, *19*(1), 1-50. https://doi.org/10.2903/j.efsa.2021.6380

European Market Observatory for Fisheries and Aquaculture Product. (2019). *The EU Fish Market 2019 edition*. Publications Office of the European Union. https://doi.org/10.2771/168390

Fano-Sizgorich, D., Vásquez-Velásquez, C., Yucra, S., Vásquez, V., Tokeshi, P., Aguilar, J., Ramírez-Atencio, C., Barr, D. B. y Gonzales, G. F. (2021). Total Urinary Arsenic and Inorganic Arsenic Concentrations and Birth Outcomes in Pregnant Women of Tacna, Peru: A Cross-Sectional Study. *Exposure and Health*, *13*(1), 133-140. https://doi.org/10.1007/s12403-020-00377-2

FAO/WHO. (2010). Report of the joint FAO / WHO expert consultation on the risks and benefits of fish consumption. En *FAO Fisheries and Acquaculture report: Vol. FIPM/R978* (Número ISSN 2070-6987). World Health Organization.

Farzan, S. F., Howe, C. G., Chavez, T. A., Hodes, T. L., Johnston, J. E., Habre, R., Dunton, G., Bastain, T. M. y Breton, C. V. (2020). Demographic predictors of urinary arsenic in a low-income predominantly Hispanic pregnancy cohort in Los Angeles. *Journal of Exposure Science and Environmental Epidemiology*, *31*(1), 91-107. https://doi.org/10.1038/s41370-020-0251-1

Ferrante, M., Napoli, S., Zuccarello, P. y Cristaldi, A. (2019). Systematic review of arsenic in fresh seafood from the Mediterranean Sea and European Atlantic coasts : A health risk assessment. *Food and chemical Toxicology*, *126*(January), 322-331. https://doi.org/10.1016/j.fct.2019.01.010

Filippini, T., Cilloni, S., Malavolti, M., Violi, F., Malagoli, C., Tesauro, M., Bottecchi, I., Ferrari, A., Vescovi, L. y Vinceti, M. (2018). Dietary intake of cadmium, chromium, copper, manganese, selenium and zinc in a Northern Italy community. *Journal*

of Trace Elements in Medicine and Biology, *50*, 508-517. https://doi.org/10.1016/j.jtemb.2018.03.001

Filippini, T., Michalke, B., Grill, P., Malagoli, C., Malavolti, M., Vescovi, L., Sieri, S., Krogh, V., Cherubini, A., Maffeis, G., Lucchini, R., Ferrante, M. y Vinceti, M. (2017). Determinants of serum manganese levels in an Italian population. *Molecular Medicine Reports*, *15*(5), 3340-3349. https://doi.org/10.3892/mmr.2017.6379

Finley, J. W. (1999). Manganese absorption and retention by young women is associated with serum ferritin concentration. *American Journal of Clinical Nutrition*, *70*(1), 37-43. https://doi.org/10.1093/ajcn/70.1.37

Flora, S. S. (2015). Arsenic: Chemistry, Occurrence, and Exposure. En S. S. Flora (Ed.), *Handbook of Arsenic Toxicology*. Elsevier.

Food and Agriculture Organization of the United Nations. (2017). *Food Balance Sheets*. FAOSTAT. http://www.fao.org/faostat/es/#data/FBS

Fowler, B. A., Chou, C. S. J., Jones, R. L., Jr, D. W. S., Chen, C., Chou, S. C., Jones, R. L., Sullivan, D. W. y Chen, C. (2015). Arsenic. En G. Nordberg, B. Fowler, & M. Nordberg (Eds.), *Handbook on the Toxicology of Metals* (Fourth Edi, Vol. 1, pp. 581-624). Elsevier. https://doi.org/10.1016/B978-0-444-59453-2.00028-7

Freeland-Graves, J. H., Mousa, T. Y. y Kim, S. (2016). International variability in diet and requirements of manganese : Causes and consequences. *Journal of Trace Elements in Medicine and Biology*, *38*, 24-32. https://doi.org/10.1016/j.jtemb.2016.05.004

Freire, C., Amaya, E., Gil, F., Fernández, M. F., Murcia, M., Llop, S., Andiarena, A., Aurrekoetxea, J., Bustamante, M., Guxens, M., Ezama, E., Fernández-Tardón, G. y Olea, N. (2018). Prenatal co-exposure to neurotoxic metals and neurodevelopment in preschool children: The Environment and Childhood (INMA) Project. *Science of The Total Environment*, *621*, 340-351. https://doi.org/10.1016/j.scitotenv.2017.11.273

Freire, C., Amaya, E., Gil, F., Murcia, M. y Llop, S. (2019). Placental metal concentrations and birth outcomes : The Environment and Childhood (INMA) project. *International Journal of Hygiene and Environmental Health*, *December 2018*, 0-1. https://doi.org/10.1016/j.ijheh.2018.12.014

Gamble, M. V., Liu, X., Ahsan, H., Pilsner, J. R., Ilievski, V., Slavkovich, V., Parvez, F., Chen, Y., Levy, D., Factor-Litvak, P. y Graziano, J. H. (2006). Folate and arsenic metabolism: A double-blind, placebo-controlled folic acid-supplementation trial in Bangladesh. *American Journal of Clinical Nutrition*, *84*(5), 1093-1101. https://doi.org/10.1093/ajcn/84.5.1093

Gamble, M. V., Liu, X., Ahsan, H., Pilsner, J. R., Illievski, V., Slavkovich, V., Parvez, F., Levy, D., Factor-Litvak, P. y Graziano, J. H. (2005). Folate, homocysteine, and arsenic metabolism in arsenic-exposed individuals in Bangladesh. *Environmental Health Perspectives*, *113*(12), 1683-1688. https://doi.org/10.1289/ehp.8084

Gamboa-Loira, B., Cebrián, M. E., Franco-Marina, F. y López-Carrillo, L. (2017). Arsenic metabolism and cancer risk : A meta-analysis. *Environmental Research*, *156*, 551-558. https://doi.org/10.1016/j.envres.2017.04.016

Ganger, R., Garla, R., Mohanty, B. P., Bansal, M. P. y Garg, M. L. (2016). Protective Effects of Zinc Against Acute Arsenic Toxicity by Regulating Antioxidant Defense System and Cumulative Metallothionein Expression. *Biological Trace Element Research*, *169*(2), 218-229. https://doi.org/10.1007/s12011-015-0400-x

Gao, S., Lin, P., Mostofa, G., Quamruzzaman, Q., Rahman, M. L. M., Rahman, M. L. M., Su, L., Hsueh, Y., Weisskopf, M., Coull, B. y Christiani, D. C. (2019). Determinants of arsenic methylation efficiency and urinary arsenic level in pregnant women in Bangladesh. *Environmental Health*, *18*(94), 1-14.

Gardner, R. M., Kippler, M., Tofail, F., Bottai, M., Hamadani, J., Grandér, M., Nermell, B., Palm, B., Rasmussen, K. M. y Vahter, M. (2013). Environmental exposure to metals and children's growth to age 5 years: A prospective cohort study. *American Journal of Epidemiology*, *177*(12), 1356-1367. https://doi.org/10.1093/aje/kws437

Gardner, R. M., Nermell, B., Kippler, M., Grandér, M., Li, L., Ekström, E. C., Rahman, A., Lönnerdal, B., Hoque, A. M. W. y Vahter, M. (2011). Arsenic methylation efficiency increases during the first trimester of pregnancy independent of folate status. *Reproductive Toxicology*, *31*(2), 210-218. https://doi.org/10.1016/j.reprotox.2010.11.002

Gimou, M.-M., Charrondière, U. R., Leblanc, J., Pouillot, R., Noël, L. y Guérin, T. (2014). 25 elements in foodstuffs in Yaounde The Cameroonian Total Diet Study. *Journal of Food Composition and Analysis*, *34*, 39-55. https://doi.org/10.1016/j.jfca.2014.02.005

Gong, L., Yang, Q., Liu, C. W. B., Wang, X. y Zeng, H. L. (2021). Assessment of 12 Essential and Toxic Elements in Whole Blood of Pregnant and Non-pregnant Women Living in Wuhan of China. *Biological Trace Element Research*, *199*(6), 2121-

2130. https://doi.org/10.1007/s12011-020-02337-y

Grandjean, P., Abdennebi-najar, L., Barouki, R., Cranor, C. F., Etzel, R. A., Gee, D., Heindel, J. J., Hougaard, K. S., Hunt, P., Nawrot, T. S., Prins, G. S., Ritz, B., Soffritti, M., Sunyer, J. y Weihe, P. (2019). Timescales of developmental toxicity impacting on research and needs for intervention. *Basic & clinical pharmacology & toxicology*, *125*(Suppl 3), 70-80. https://doi.org/10.1111/bcpt.13162.Timescales

Grandjean, P. y Landrigan, P. J. (2006). Developmental neurotoxicity of industrial chemicals. *The Lancet*, *368*, 2167-2178. https://doi.org/10.1016/S0140-6736(06)69665-7

Grandjean, P. y Landrigan, P. J. (2014). Neurobehavioural effects of developmental toxicity. *The Lancet Neurology*, *13*, 330-338. https://doi.org/10.1016/S1474-4422(13)70278-3

Gribble, M. O., Voruganti, V. S., Cole, S. A., Haack, K., Balakrishnan, P., Laston, S. L., Tellez-Plaza, M., Francesconi, K. A., Goessler, W., Umans, J. G., Thomas, D. C., Gilliland, F., North, K. E., Franceschini, N. y Navas-Acien, A. (2015). Linkage Analysis of Urine Arsenic Species Patterns in the Strong Heart Family Study. *Toxicological Sciences*, *148*(1), 89-100. https://doi.org/10.1093/toxsci/kfv164

Grilo, L. F., Tocantins, C., Diniz, M. S., Gomes, R. M., Oliveira, P. J., Matafome, P. y Pereira, S. P. (2021). Metabolic Disease Programming: From Mitochondria to Epigenetics, Glucocorticoid Signalling and Beyond. *European Journal of Clinical Investigation*, *51*(10), 1-21. https://doi.org/10.1111/eci.13625

Gundacker, C., Neesen, J., Straka, E., Ellinger, I., Dolznig, H. y Hengstschläger, M. (2016). Genetics of the human placenta: implications for toxicokinetics. *Archives of Toxicology*, *90*(11), 2563-2581. https://doi.org/10.1007/s00204-016-1816-6

Gunier, R. B., Arora, M., Jerrett, M., Bradman, A., G, H. K., Mora, A. M., Kogut, K., Hubbard, A., Austin, C., Holland, N. y Eskenazi, B. (2015). Manganese in Teeth and Neurodevelopment in Young Mexican- American Children. *Environmental Research*, *142*, 688-695. https://doi.org/10.1111/ajt.13087.TIM-1

Guxens, M., Ballester, F., Espada, M., Ferna, M. F., Grimalt, J. O., Ibarluzea, J., Olea, N., Rebagliato, M., Tardón, A., Torrent, M., Vioque, J., Vrijheis, M., Sunyer, J. y on behalf of the INMA project. (2012). Cohort Profile : The INMA — INfancia y Medio Ambiente —(Environment and Childhood) Project. *International Journal of Epidemiology*, *41*, 930-940. https://doi.org/10.1093/ije/dyr054

Hall, M., Gamble, M., Slavkovich, V., Liu, X., Levy, D., Cheng, Z., van Geen, A., Yunus, M., Rahman, M., Pilsner, J. R. y Graziano, J. (2007). Determinants of arsenic metabolism: Blood arsenic metabolites, plasma folate, cobalamin, and homocysteine concentrations in maternal-newborn pairs. *Environmental Health Perspectives*, *115*, 1503-1509. https://doi.org/10.1289/ehp.9906

Hamadani, J. D., Tofail, F., Nermell, B., Gardner, R. M., Shiraji, S., Bottai, M., Arifeen, S. El, Huda, S. N. y Vahter, M. (2011). Critical windows of exposure for arsenic-associated impairment of cognitive function in pre-school girls and boys: a population-based cohort study. *International Journal of Epidemiology*, *40*(6), 1593-1604. https://doi.org/10.1093/ije/dyr176

Hayakawa, T., Kobayashi, Y. y Cui, X. (2005). A new metabolic pathway of arsenite : arsenic – glutathione complexes are substrates for human arsenic methyltransferase Cyt19. *Archives of Toxicology*, *79*, 183-191. https://doi.org/10.1007/s00204-004-0620-x

Heck, J. E., Gamble, M. V., Chen, Y., Graziano, J. H., Slavkovich, V., Parvez, F., Baron, J. A., Howe, G. R. y Ahsan, H. (2007). Consumption of folate-related nutrients and metabolism of arsenic in Bangladesh. *American Journal of Clinical Nutrition*, *85*(5), 1367-1374. https://doi.org/10.1093/ajcn/85.5.1367

Heindel, J. J., Balbus, J., Birnbaum, L., Brune-Drisse, M. N., Grandjean, P., Gray, K., Landrigan, P. J., Sly, P. D., Suk, W., Slechta, D. C., Thompson, C. y Hanson, M. (2015). Developmental origins of health and disease: Integrating environmental influences. *Endocrinology*, *156*(10), 3416-3421. https://doi.org/10.1210/EN.2015-1394

Heindel, J. J., Skalla, L. A., Joubert, B. R., Dilworth, C. H. y Gray, K. A. (2017). Review of developmental origins of health and disease publications in environmental epidemiology. *Reproductive Toxicology*, *68*, 34-48. https://doi.org/10.1016/j.reprotox.2016.11.011

Hopenhayn-Rich, C., Biggs, M. Lou, Smith, A. H., Kalman, D. A. y Moore, L. E. (1996). Methylation Study of a Population Environmentally Exposed to Arsenic in Drinking Water. *Environmental Health Perspectives*, *104*(6), 620-628. https://doi.org/10.2307/3433091

Hopenhayn, C., Huang, B., Christian, J., Peralta, C., Ferreccio, C., Atallah, R. y Kalman, D. (2003). Profile of urinary arsenic metabolites during pregnancy. *Environmental Health Perspectives*, *111*(16), 1888-1891. https://doi.org/10.1289/ehp.6254

Howe, C. G., Farzan, S. F., Garcia, E., Jursa, T., Iyer, R., Berhane, K., Chavez, T. A., Hodes, T. L., Grubbs, B. H., Funk, W. E., Smith, D. R., Bastain, T. M. y Breton, C. V. (2020). Arsenic and birth outcomes in a predominately lower income Hispanic pregnancy cohort in Los Angeles. *Environmental Research*, *184*, 109294. https://doi.org/10.1016/j.envres.2020.109294

Howe, C. G., Li, Z., Zens, M. S., Palys, T., Chen, Y., Channon, J. Y., Karagas, M. R. y Farzan, S. F. (2017). Dietary B vitamin intake is associated with lower urinary monomethyl arsenic and oxidative stress marker 15-F2t-isoprostane among New Hampshire adults. *The Journal of Nutrition*, *147*(12), 2289-2296. https://doi.org/10.3945/jn.117.253419

Howe, C. G., Niedzwiecki, M. M., Hall, M. N., Liu, X., Ilievski, V., Slavkovich, V., Alam, S., Siddique, A. B., Graziano, J. H. y Gamble, M. V. (2014). Folate and cobalamin modify associations between S-adenosylmethionine and methylated arsenic metabolites in arsenic-exposed bangladeshi adults. *The Journal of Nutrition*, *144*(5), 690-697. https://doi.org/10.3945/jn.113.188789

Hsieh, R. L., Huang, Y. L., Shiue, H. S., Huang, S. R., Lin, M. I., Mu, S. C., Chung, C. J. y Hsueh, Y. M. (2014). Arsenic methylation capacity and developmental delay in preschool children in Taiwan. *International Journal of Hygiene and Environmental Health*, *217*(6), 678-686. https://doi.org/10.1016/j.ijheh.2014.02.004

Hsueh, Y. M., Chen, W. J., Lee, C. Y., Chien, S. N., Shiue, H. S., Huang, S. R., Lin, M. I., Mu, S. C. y Hsieh, R. L. (2016). Association of Arsenic Methylation Capacity with Developmental Delays and Health Status in Children: A Prospective Case-Control Trial. *Scientific Reports*, *6*, 37287. https://doi.org/10.1038/srep37287

Hu, J., Wu, C., Zheng, T., Zhang, B., Xia, W., Peng, Y., Liu, W., Jiang, M., Liu, S., Buka, S. L., Zhou, A., Zhang, Y., Jiang, Y., Hu, C., Chen, X., Zeng, Q., Chen, X., Xu, B., Zhang, X., … Xu, S. (2018). Critical windows for associations between manganese exposure during pregnancy and size at birth: A longitudinal cohort study in Wuhan, China. *Environmental Health Perspectives*, *126*(12), 1-9. https://doi.org/10.1289/EHP3423

Huang, L., Wu, H. y Van Der Kuijp, T. J. (2015). The health effects of exposure to arsenic-contaminated drinking water: A review by global geographical distribution. *International Journal of Environmental Health Research*, *25*(4), 432-452. https://doi.org/10.1080/09603123.2014.958139

Ijomone, O. M., Ijomone, O. K., Iroegbu, J. D., Ifenatuoha, C. W., Olung, N. F. y Aschner, M. (2020). Epigenetic influence of environmentally neurotoxic metals. *NeuroToxicology*, *81*, 51-65. https://doi.org/10.1016/j.neuro.2020.08.005

Iniguez, C., Ballester, F., Estarlich, M., Llop, S., Fernandez-Patier, R., Aguirre-Alfaro, A. y Esplugues, A. (2009). Estimation of personal NO2 exposure in a cohort of pregnant women. *Science of The Total Environment*, *407*(23), 6093-6099. https://doi.org/10.1016/j.scitotenv.2009.08.006

Institute of Medicine. (2006). Dietary Reference Intakes: The Essential Guide to Nutrient Requirements (2006). En *Dietary Reference Intakes: the essential guide to nutrient requirements*. National Academies Press (US). https://doi.org/10.17226/11537

Instituto Nacional de Seguridad Salud y Bienestar en el Trabajo. (2018). *Límites de Exposición Profesional para Agentes Químicos en España 2018* (Instituto Nacional de Seguridad Salud y Bienestar en el Trabajo (ed.)).

International Agency for Research on Cancer. (2012). Arsenic, metals, fibres and dusts. IARC monographs on the evaluation of carcinogenic risks to humans. En *Iarc Monographs* (Vol. 100). International Agency for Research on Cancer.

Irizar, A., Molinuevo, A., Andiarena, A., Jimeno-Romero, A., San Román, A., Broberg, K., Llop, S., Soler-Blasco, R., Murcia, M., Ballester, F. y Lertxundi, A. (2021). Prenatal manganese serum levels and neurodevelopment at 4 years of age. *Environmental Research*, *197*, 111172.

Jansen, R. J., Argos, M., Tong, L., Li, J., Rakibuz-Zaman, Muammad Islam, T., Slavkovich, V., Ahmed, A., Navas-Acien, A., Parvez, F., Yu, C., Mary, G., Graziano, J. H., Pierce, B. L. y Ahsan, H. (2016). Determinants and consequences of arsenic metabolism efficiency among 4,794 individuals: demographics, lifestyle, genetics, and toxicity. *Cancer epidemiology, biomarkers & prevention*, *25*(2), 381-390. https://doi.org/10.1016/j.cogdev.2010.08.003.Personal

Jiang, Y. y Zheng, W. (2005). Cardiovascular toxicities upon manganese exposure. *Cardiovascular Toxicology*, *5*(4), 345-354. https://doi.org/10.1385/ct:5:4:345

Joint FAO/WHO Expert Committee on Food Additives. (2011). *Safety evaluation of certain contaminants in food.WHO technical report series: 959*. World Health Organization.

Jones, M. R., Tellez-Plaza, M., Vaidya, D., Grau, M., Francesconi, K. A., Goessler, W., Guallar, E., Post, W. S., Kaufman, J. D. y Navas-Acien, A. (2016). Estimation of Inorganic Arsenic Exposure in Populations with Frequent Seafood Intake: Evidence from MESA and NHANES. *American Journal of Epidemiology*, *184*(8), 590-602. https://doi.org/10.1093/aje/kww097

Julvez, J., Forns, M., Ribas-Fitó, N., Torrent, M. y Sunyer, J. (2011). Attention behavior and hyperactivity and concurrent neurocognitive and social competence functioning in 4-year-olds from two population-based birth cohorts. *European Psychiatry*, *26*, 381-389. https://doi.org/10.1016/j.eurpsy.2010.03.013

Julvez, J., Méndez, M., Fernandez-Barres, S., Romaguera, D., Vioque, J., Llop, S., Ibarluzea, J., Guxens, M., Avella-Garcia, C., Tardón, A., Riaño, I., Andiarena, A., Robinson, O., Arija, V., Esnaola, M., Ballester, F. y Sunyer, J. (2016). Maternal consumption of seafood in pregnancy and child neuropsychological development: A longitudinal study based on a population with high consumption levels. *American Journal of Epidemiology*, *183*(3), 169-182. https://doi.org/10.1093/aje/kwv195

Karagas, M., Gossai, A., Pierce, B. y Ahsan, H. (2015). Drinking Water Arsenic Contamination, Skin Lesions, and Malignancies: A Systematic Review of the Global Evidence. *Current Environmental Health Reports*, *2*(1), 52-68. https://doi.org/10.1007/s40572-014-0040-x.Drinking

Karmaus, W., Ziyab, A. H. y Mukherjee, N. (2019). Epigenetics: Strategies for Prevention Research. En *Health Impacts of developmental Exposure to Environmentan Chemicals*. Springer.

Kim, Y. (2018). Sex, pregnancy, and age-specific differences of blood manganese levels in relation to iron status; what does it mean? *Toxicology Reports*, *5*(November 2017), 28-30. https://doi.org/10.1016/j.toxrep.2017.12.003

Kim, Y. y Park, S. (2014). Iron deficiency increases blood concentrations of neurotoxic metals in children. *Korean Journal of Pediatrics*, *57*(8), 345. https://doi.org/10.3345/kjp.2014.57.8.345

Kordas, K., Queirolo, E. I., Peregalli, F., Hsiao, P. Y., Lu, Y., Vahter, M., Institutet, K., Mañay, N., Peregalli, F., Hsiao, P. Y., Lu, Y. y Vahter, M. (2016). Low-level arsenic exposure: Nutritional and dietary predictors in first-grade Uruguayan children. *Environmental Research*, *147*, 16-23. https://doi.org/10.1016/j.envres.2016.01.022

Kordas, K., Roy, A., López, P., García-Vargas, G., Cebrián, M. E., Vera-Aguilar, E. y Rosado, J. L. (2017). Iron and Zinc Supplementation Does Not Impact Urinary Arsenic Excretion in Mexican School Children. *The Journal of Pediatrics*, *185*, 205-210. https://doi.org/10.1016/j.jpeds.2017.02.040

Kumar, A., Malhotra, A., Nair, P., Garg, M. L. y Dhawan, D. K. (2010). Protective role of zinc in ameliorating arsenic-induced oxidative stress and histological changes in rat liver. *Journal of Environmental Pathology, Toxicology and Oncology*, *29*(2), 91-100. https://doi.org/10.1615/JEnvironPatholToxicolOncol.v29.i2.30

Kuo, C., Moon, K. A., Wang, S., Silbergeld, E. y Navas-acien, A. (2017). The Association of Arsenic Metabolism with Cancer , Cardiovascular Disease , and Diabetes : A Systematic Review of the Epidemiological Evidence. *Environmental Health Perspectives*, *125*(8).

Kupsco, A., Estrada-gutierrez, G., Cantoral, A., Schnaas, L., Pantic, I., Amarasiriwardena, C., Svensson, K., Bellinger, D. C., Téllez-Rojo, M. M., Baccarelli, A. A. y Wright, R. O. (2020). Modification of the effects of prenatal manganese exposure on child neurodevelopment by maternal anemia and iron deficiency. *Pediatric research*, *88*(2), 325-333. https://doi.org/10.1038/s41390-020-0754-4.Modification

Kurzius-Spencer, M., Thomson, C. A., Hartz, V., Hsu, C., Burgess, J. L., Kay, M., Rourke, O. y Harris, R. B. (2017). Nutrients in one-carbon metabolism and urinary arsenic methylation in the National Health and Nutrition Examination Survey (NHANES) 2003–2004. *Science of the Total Environment*, *607-608*, 381-390. https://doi.org/10.1016/j.scitotenv.2017.07.019

Laine, J. E., Bailey, K. A., Rubio-andrade, M., Olshan, A. F., Smeester, L., Drobná, Z., Herring, A. H., Stýblo, M., García-Vargas, G. G. y Fry, R. C. (2015). Maternal Arsenic Exposure , Arsenic Methylation Efficiency, and Birth Outcomes in the Biomarkers of Exposure to ARsenic (BEAR) Pregnancy Cohort in Mexico. *Environmental health Perspectives*, *123*(2), 186-192.

Laine, J. E., Ilievski, V., Richardson, D. B., Herring, A. H., Stýblo, M., Rubio-Andrade, M., Garcia-Vargas, G., Gamble, M. V. y Fry, R. C. (2018). Maternal one carbon metabolism and arsenic methylation in a pregnancy cohort in Mexico. *Journal of Exposure Science and Environmental Epidemiology*, *28*, 505-514. https://doi.org/10.1038/s41370-018-0041-1

Lee, J. J., Valeri, L., Kapur, K., Ibne Hasan, M. O. S., Quamruzzaman, Q., Wright, R. O., Bellinger, D. C., Christiani, D. C. y Mazumdar, M. (2018). Growth parameters at birth mediate the relationship between prenatal manganese exposure and cognitive test scores among a cohort of 2- to 3-year-old Bangladeshi children. *International Journal of Epidemiology*, *03*(August), 3. https://doi.org/10.1093/ije/dyy069

Lee, K. S., Kim, K. N., Ahn, Y. D., Choi, Y. J., Cho, J., Jang, Y., Lim, Y. H., Kim, J. I., Shin, C. H., Lee, Y. A., Kim, B. N. y Hong, Y. C. (2021). Prenatal and postnatal exposures to four metals mixture and IQ in 6-year-old children: A prospective cohort study in South Korea. *Environment International*, *157*, 106798. https://doi.org/10.1016/j.envint.2021.106798

Lee, P. H. (2014). Is a Cutoff of 10% Appropriate for the Change-in-Estimate Criterion of Confounder Identification? *Journal of Epidemiology*, *24*(2), 161-167. https://doi.org/10.2188/jea.JE20130062

Leonhard, M. J., Chang, E. T., Loccisano, A. E. y Garry, M. R. (2019). A systematic literature review of epidemiologic studies of developmental manganese exposure and neurodevelopmental outcomes. *Toxicology*, *420*(September 2018), 46-65. https://doi.org/10.1016/j.tox.2019.03.004

Lertxundi, A., Baccini, M., Lertxundi, N., Fano, E., Aranbarri, A., Martínez, M. D., Ayerdi, M., Álvarez, J., Santa-Marina, L., Dorronsoro, M. y Ibarluzea, J. (2015). Exposure to fine particle matter, nitrogen dioxide and benzene during pregnancy and cognitive and psychomotor developments in children at 15months of age. *Environment International*, *80*, 33-40. https://doi.org/10.1016/j.envint.2015.03.007

Levi, M., Hjelm, C., Harari, F. y Vahter, M. (2018). ICP-MS measurement of toxic and essential elements in human breast milk . A comparison of alkali dilution and acid digestion sample preparation methods. *Clinical Biochemistry*, *53*(December 2017), 81-87. https://doi.org/10.1016/j.clinbiochem.2017.12.003

Lewis, A. S., Reid, K. R., Pollock, M. C. y Campleman, S. L. (2012). Speciated arsenic in air: Measurement methodology and risk assessment considerations. *Journal of the Air and Waste Management Association*, *62*(1), 2-17. https://doi.org/10.1080/10473289.2011.608620

Li, L., Ekström, E. C., Goessler, W., Lönnerdal, B., Nermell, B., Yunus, M., Rahman, A., El Arifeen, S., Persson, L. Å. y Vahter, M. (2008). Nutritional status has marginal influence on the metabolism of inorganic arsenic in pregnant Bangladeshi women. *Environmental Health Perspectives*, *116*(3), 315-321. https://doi.org/10.1289/ehp.10639

Liang, C., Wu, X., Huang, K., Yan, S., Li, Z., Xia, X., Pan, W., Sheng, J., Tao, R., Tao, Y., Xiang, H., Hao, J., Wang, Q., Tong, S. y Tao, F. (2020). Domain- and sex-specific effects of prenatal exposure to low levels of arsenic on children's development at 6 months of age: Findings from the Ma'anshan birth cohort study in China. *Environment International*, *135*, 105112. https://doi.org/10.1016/j.envint.2019.105112

Lin, C.-C., Chen, Y.-C., Su, F.-C., Lin, C.-M., Liao, H.-F., Hwang, Y.-H., Hsieh, W.-S., Jeng, S.-F., Su, Y.-N. y Chen, P.-C. (2013). In utero exposure to environmental lead and manganese and neurodevelopment at 2 years of age. *Environmental Research*, *123*, 52-57. https://doi.org/10.1016/J.ENVRES.2013.03.003

Liu, K. L., Tsai, T. L., Tsai, W. C., Tsai, S. F., Lee, C. H. y Wang, S. L. (2021). Prenatal heavy metal exposure, total immunoglobulin E, trajectory, and atopic diseases: A 15-year follow-up study of a Taiwanese birth cohort. *Journal of Dermatology*, *48*(10), 1542-1549. https://doi.org/10.1111/1346-8138.16058

Liu, S., Guo, X., Zhang, X., Cui, Y., Zhang, Y. y Wu, B. (2013). Impact of iron precipitant on toxicity of arsenic in water: A combined in vivo and in vitro study. *Environmental Science and Technology*, *47*, 3432-3438. https://doi.org/10.1021/es400176m

Liu, W., Xin, Y., Li, Q., Shang, Y., Ping, Z., Min, J., Cahill, C. M., Rogers, J. T. y Wang, F. (2020). Biomarkers of environmental manganese exposure and associations with childhood neurodevelopment: A systematic review and meta-analysis. *Environmental Health*, *19*, 1-22. https://doi.org/10.1186/s12940-020-00659-x

Ljung, K., Palm, B., Grandér, M. y Vahter, M. (2011). High concentrations of essential and toxic elements in infant formula and infant foods - A matter of concern. *Food Chemistry*, *127*(3), 943-951. https://doi.org/10.1016/j.foodchem.2011.01.062

Lucchini, R. G., Aschner, M., Bellinger, D. C. y Caito, S. W. (2015). Neurotoxicology of Metals. En G. Nordberg, B. Fowler, & M. Nordberg (Eds.), *Handbook on the Toxicology of Metals* (Fourth Edi, pp. 299-311). Elsevier. https://doi.org/10.1016/B978-0-08-100601-6.01329-6

Lucchini, R. G., Aschner, M., Kim, Y. y Šarić, M. (2015). Manganese. En G. Nordberg, B. Fowler, & M. Nordberg (Eds.), *Handbook on the Toxicology of Metals* (Fourth Edi, Vol. 1, pp. 975-1011). Elsevier. https://doi.org/10.1016/B978-0-444-59453-2.00045-7

Lucchini, R., Placidi, D., Cagna, G., Fedrighi, C., Oppini, M., Peli, M. y Zoni, S. (2017). Manganese and Developmental Neurotoxicity. En *Advances in Neurobiology* (Vol. 18, pp. 13-34). https://doi.org/10.1007/978-3-319-60189-2_2

Luo, J. y Shu, W. (2015). Arsenic-Induced Developmental Neurotoxicity. En S. S. Flora (Ed.), *Hanbook of Arsenic Toxicology*. Elsevier.

Marín, S., Pardo, O., Sanchez, A., Sanchis, Y., Vélez, D., Devesa, V., Font, G. y Yusà, V. (2018). Assessment of metal levels in foodstuffs from the Region of Valencia Region (Spain). *Toxicology Reports*, *5*(May), 654-670. https://doi.org/10.1016/J.TOXREP.2018.05.005

Masjosthusmann, S., Tigges, J. y Fritsche, E. (2021). Arsenic-mediated developmental neurotoxicity : Recent advances in understanding the adverse outcomes and underlying mechanisms. En M. Aschner & L. G. Costa (Eds.), *Neurotoxicity of Metals: Old Issues and New Developments* (1.ª ed., Vol. 5, pp. 35-80). Elsevier Inc. https://doi.org/10.1016/bs.ant.2020.12.002

McCarthy, D. (2009). *MSCA. Escalas McCarthy de Aptitudes y Psicomotricidad para Niños.* (T. Ediciones (ed.)). TEA Ediciones.

McRae, N., Gennings, C., Rivera Rivera, N., Tamayo-Ortiz, M., Pantic, I., Amarasiriwardena, C., Schnaas, L., Wright, R., Tellez-Rojo, M. M., Wright, R. O. y Rosa, M. J. (2022). Association between prenatal metal exposure and adverse respiratory symptoms in childhood. *Environmental Research*, *205*(November 2021), 112448. https://doi.org/10.1016/j.envres.2021.112448

Meharg, A. A. y Zhao, F.-J. (2012). *Arsenic and Rice*.

Miah, M. R., Ijomone, O. M., Okoh, C. O. ., Ijomone, O. K., Akingbade, G. T., Ke, T., Krum, B., da Cunha Martins, A., Akinyemi, A., Aranoff, N., Antunes Soares, F. A., Bowman, A. B. y Aschner, M. (2020). The effects of manganese overexposure on brain health. *Neurochemistry International*, *135*, 104688. https://doi.org/10.1016/j.neuint.2020.104688

Ministerio de Sanidad Servicios Sociales e Igualdad. (2019a). *Calidad del Agua de Consumo Humano en España 2019. Anexo: Tablas*. Ministerio de sanidad servicios sociales e igualdad.

Ministerio de Sanidad Servicios Sociales e Igualdad. (2019b). *Calidad del agua de consumo humano en España 2019 Anexo III: Mapas*. Ministerio de sanidad servicios sociales e igualdad.

Mishra, D. y Flora, S. (2008). Differential oxidative stress and DNA damage in rat brain regions and blood following chronic arsenic exposure. *Toxicology and Industrial Health*, *24*(4), 247-256. https://doi.org/10.1177/0748233708093355

Mitchell, E. J., Frisbie, S. H., Roudeau, S., Carmona, A. y Ortega, R. (2020). Estimating daily intakes of manganese due to breast milk, infant formulas, or young child nutritional beverages in the United States and France: Comparison to sufficiency and toxicity thresholds. *Journal of Trace Elements in Medicine and Biology*, *62*(February), 126607. https://doi.org/10.1016/j.jtemb.2020.126607

Miyashita, C., Saijo, Y., Ito, Y., Ikeda-Araki, A., Itoh, S., Yamazaki, K., Kobayashi, S., Ait Bamai, Y., Masuda, H., Tamura, N., Itoh, M., Yamaguchi, T., Yamazaki, S. y Kishi, R. (2021). Association between the concentrations of metallic elements in maternal blood during pregnancy and prevalence of abdominal congenital malformations: The japan environment and children's study. *International Journal of Environmental Research and Public Health*, *18*(19). https://doi.org/10.3390/ijerph181910103

Mochizuki, H. (2019). Arsenic Neurotoxicity in Humans. *International Journal of Molecular Sciences*, *20*, 3418. https://doi.org/https://doi.org/10.3390/ijms20143418

Molin, M., Ulven, S. M., Dahl, L., Telle-Hansen, V. H., Holck, M., Skjegstad, G., Ledsaak, O., Sloth, J. J., Goessler, W., Oshaug, A., Alexander, J., Fliegel, D., Ydersbond, T. A. y Meltzer, H. M. (2012). Humans seem to produce arsenobetaine and dimethylarsinate after a bolus dose of seafood. *Environmental Research*, *112*, 28-39. https://doi.org/10.1016/j.envres.2011.11.007

Moon, K. A., Oberoi, S., Barchowsky, A., Chen, Y., Guallar, E., Nachman, K. E., Rahman, M., Sohel, N., D'Ippoliti, D., Wade, T. J., James, K. A., Farzan, S. F., Karagas, M. R., Ahsan, H. y Navas-Acien, A. (2017). A dose-response meta-analysis of chronic arsenic exposure and incident cardiovascular disease. *International Journal of Epidemiology*, *46*(6), 1924-1939. https://doi.org/10.1093/ije/dyx202

Moon, K., Guallar, E. y Navas-Acien, A. (2012). Arsenic Exposure and Cardiovascular Disease: An Updated Systematic Review. *Current atherosclerosis reports*, *14*(6), 542-555. https://doi.org/doi:10.1007/s11883-012-0280-x

Mora, A. M., Arora, M., Harley, K. G., Kogut, K., Parra, K., Hernández-Bonilla, D., Gunier, R. B., Bradman, A., Smith, D. R. y Eskenazi, B. (2015). Prenatal and postnatal manganese teeth levels and neurodevelopment at 7, 9, and 10.5 years in the CHAMACOS cohort. *Environmental international*, *84*, 39-54. https://doi.org/10.1016/j.envint.2015.07.009.Prenatal

Mora, A. M., Córdoba, L., Cano, J. C., Hernandez-Bonilla, D., Pardo, L., Schnaas, L., Smith, D. R., Menezes-Filho, J. A., Mergler, D., Lindh, C. H., Eskenazi, B., Van, B. y De Joode, W. (2018). Prenatal Mancozeb Exposure, Excess Manganese, and Neurodevelopment at 1 Year of Age in the Infants' Environmental Health (ISA) Study. *Enviromental Health Perspectives*, *126*(May), 1-9. https://doi.org/10.1289/EHP1955

Moradnia, M., Movahedian Attar, H., Heidari, Z., Mohammadi, F. y Kelishadi, R. (2021). Monitoring of urinary arsenic (As) and lead (Pb) among a sample of pregnant Iranian women. *Journal of Environmental Health Science and Engineering*, *19*(2), 1901-1909. https://doi.org/10.1007/s40201-021-00743-5

Mouhamed, D. H., Ezzaher, A., Neffati, F., Douki, W. y Najjar, M. F. (2011). Effect of cigarette smoking on plasma homocysteine concentrations. *Clinical Chemistry and Laboratory Medicine*, *49*(3), 479-483. https://doi.org/10.1515/CCLM.2011.062

Muñoz-Rocha, T. V., Tamayo y Ortiz, M., Romero, M., Pantic, I., Schnaas, L., Bellinger, D., Claus-Henn, B., Wright, R., Wright, R. O. y Téllez-Rojo, M. M. (2018). Prenatal co-exposure to manganese and depression and 24-months neurodevelopment. *NeuroToxicology*, *64*, 134-141. https://doi.org/10.1016/j.neuro.2017.07.007

Myren, M., Mose, T., Mathiesen, L. y Knudsen, L. E. (2007). The human placenta - An alternative for studying foetal exposure. *Toxicology in Vitro*, *21*(7), 1332-1340. https://doi.org/10.1016/j.tiv.2007.05.011

Nandakumaran, M., Al-Sannan, B., Al-Sarraf, H. y Al-Shammari, M. (2016). Maternal – fetal transport kinetics of manganese in perfused human placental lobule in vitro. *The Journal of Maternal-Fetal & Neonatal Medicine*, *29*(2), 274-278. https://doi.org/10.3109/14767058.2014.998193

Navas-Acien, A., Francesconi, K. A., Silbergeld, E. K. y Guallar, E. (2011). Seafood Intake and Urine Concentrations of Total Arsenic, Dimethylarsinate and Arsenobetaine in the US Population. *Environmental Research*, *111*(1), 110-118. https://doi.org/10.1016/j.envres.2010.10.009

Nesnow, S., Roop, B. C., Lambert, G., Kadiiska, M., Mason, R. P., Cullen, W. R. y Mass, M. J. (2002). DNA damage induced by methylated trivalent arsenicals is mediated by reactive oxygen species. *Chemical Research in Toxicology*, *15*, 1627-1634. https://doi.org/10.1021/tx025598y

Nocoń, K., Rogula-Kozłowska, W., Majewski, G. y Rogula-Kopiec, P. (2020). Soluble inorganic arsenic species in atmospheric submicron particles in two polish urban background sites. *Sustainability (Switzerland)*, *12*(3). https://doi.org/10.3390/su12030837

Nordberg, G. F., Jin, T., Hong, F., Zhang, A., Buchet, J. y Bernard, A. (2005). Biomarkers of cadmium and arsenic interactions. *Toxicology and Applied Pharmacology*, *206*(2), 191-197. https://doi.org/10.1016/J.TAAP.2004.11.028

Nyanza, E. C., Bernier, F. P., Martin, J. W., Manyama, M., Hatfield, J. y Dewey, D. (2021). Effects of prenatal exposure and co-exposure to metallic or metalloid elements on early infant neurodevelopmental outcomes in areas with small-scale gold mining activities in Northern Tanzania. *Environment International*, *149*, 106104. https://doi.org/10.1016/j.envint.2020.106104

Nyarko-Danquah, I., Pajarillo, E., Digman, A., Soliman, K. F. A., Aschner, M. y Lee, E. (2020). Manganese Accumulation in the Brain via Various Transporters and Its Neurotoxicity Mechanisms. *Molecules*, *25*, 5880. https://doi.org/10.3390/molecules25245880

O'Bryant, S. E., Edwards, M., Menon, C. V., Gong, G. y Barber, R. (2011). Long-term low-level arsenic exposure is associated with poorer neuropsychological functioning: A project FRONTIER study. *International Journal of Environmental Research and Public Health*, *8*, 861-874. https://doi.org/10.3390/ijerph8030861

O´Neal, S. L. y Zheng, W. (2015). Manganese Toxicity Upon Overexposure: a Decade in Review. *Current Environmental Health Reports*, *2*(3), 315-328. https://doi.org/10.1007/s40572-015-0056-x.Manganese

Ode, A., Rylander, L., Gustafsson, P., Lundh, T., Källén, K., Olofsson, P., Ivarsson, S. A. y Rignell-Hydbom, A. (2015). Manganese and selenium concentrations in umbilical cord serum and attention deficit hyperactivity disorder in childhood. *Environmental Research*, *137*, 373-381. https://doi.org/10.1016/j.envres.2015.01.001

Olusanya, B. O., Davis, A. C., Wertlieb, D., Boo, N. Y., Nair, M. K. C., Halpern, R., Kuper, H., Breinbauer, C., de Vries, P. J., Gladstone, M., Halfon, N., Kancherla, V., Mulaudzi, M. C., Kakooza-Mwesige, A., Ogbo, F. A., Olusanya, J. O., Williams,

A. N., Wright, S. M., Manguerra, H., … Kassebaum, N. J. (2018). Developmental disabilities among children younger than 5 years in 195 countries and territories, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. *The Lancet Global Health*, *6*, e1100-e1121. https://doi.org/10.1016/S2214-109X(18)30309-7

Oppenheimer, A. V, Bellinger, D. C., Coull, B. A., Weisskopf, M. G. y Korrick, S. A. (2022). Prenatal exposure to chemical mixtures and working memory among adolescents. *Environmental Research*, *205*, 112436. https://doi.org/10.1016/j.envres.2021.112436

Otero, X. L., Atiaga, O., Estrella, R., Tierra, W., Ruales, J., Zayas, L., Souza, V., Ferreira, T. O., Nóbrega, G. N., Oliveira, D. P., Queiroz, H. M. y Nunes, L. M. (2020). Geographical variations in arsenic contents in rice plants from Latin America and the Iberian Peninsula in relation to soil conditions. *Environmental Geochemistry and Health*, *42*(10), 3351-3372. https://doi.org/10.1007/s10653-020-00581-8

Oulhote, Y. y Bellinger, D. C. (2019). Neurodevelopment and Neurobehavioral Disorders in Relation to Developmental Exposures. En R. Kishi & P. Grandjean (Eds.), *Health Impacts of Developmental Exposure to Environmental Chemicals*. Springer.

Oulhote, Y., Mergler, D., Barbeau, B., Bellinger, D. C., Bouffard, T., Brodeur, M.-È., Saint-Amour, D., Legrand, M., Sauvé, S. y Bouchard, M. F. (2014). Neurobehavioral Function in School-Age Children Exposed to Manganese in Drinking Water. *Environmental Health Perspectives*, *122*(12), 1343-1350. https://doi.org/10.1289/ehp.1307918

Pajarillo, E., Nyarko-danquah, I. y Adinew, G. (2021). Neurotoxicity mechanisms of manganese in the central nervous system. En M. Aschner & L. G. Costa (Eds.), *Neurotoxicity of Metals: Old Issues and New Developments* (1.ª ed., Vol. 5, pp. 215-238). Elsevier Inc. https://doi.org/10.1016/bs.ant.2020.11.003

Palau Miguel, M., Ligia Guevara Alemany, E. y Moreno Seisdedos, M. (2008). *Calidad de agua de consumo humano en España. Informe técnico. Trienio 2005-2006-2007*.

Palau Miguel, M., Ligia Guevara Alemany, E. y Moreno Seisdedos, M. (2010). *Calidad del agua de consumo humano en España Informe técnico. Año 2008*.

Palma, I., Farran, A. y Cervera, P. (2008). *Tables of Food Composition for household measures of habitual consumption in Spain: CESNID* (4.ª ed.). McGraw Hill.

Parajuli, R. P., Fujiwara, T., Umezaki, M., Furusawa, H. y Watanabe, C. (2014). Home environment and prenatal exposure to lead, arsenic and zinc on the neurodevelopment of six-month-old infants living in Chitwan Valley, Nepal. *Neurotoxicology and Teratology*, *41*, 89-95. https://doi.org/10.1016/j.ntt.2013.12.006

Parajuli, R. P., Fujiwara, T., Umezaki, M. y Watanabe, C. (2013). Association of cord blood levels of lead, arsenic, and zinc with neurodevelopmental indicators in newborns: A birth cohort study in Chitwan Valley, Nepal. *Environmental Research*, *121*, 45-51. https://doi.org/10.1016/J.ENVRES.2012.10.010

Parajuli, R. P., Fujiwara, T., Umezaki, M. y Watanabe, C. (2015). Home environment and cord blood levels of lead, arsenic, and zinc on neurodevelopment of 24 months children living in Chitwan Valley, Nepal. *Journal of Trace Elements in Medicine and Biology*, *29*, 315-320. https://doi.org/10.1016/J.JTEMB.2014.08.006

Parajuli, R. P., Umezaki, M., Fujiwara, T. y Watanabe, C. (2015). Association of Cord Blood Levels of Lead, Arsenic, and Zinc and Home Environment with Children Neurodevelopment at 36 Months Living in Chitwan Valley, Nepal. *PLOS ONE*, *10*(3), e0120992. https://doi.org/10.1371/journal.pone.0120992

Parvez, F., Wasserman, G. A., Factor-Litvak, P., Liu, X., Slavkovich, V., Siddique, A. B., Sultana, R., Sultana, R., Islam, T., Levy, D., Mey, J. L., van Geen, A., Khan, K., Kline, J., Ahsan, H. y Graziano, J. H. (2011). Arsenic exposure and motor function among children in Bangladesh. *Environmental Health Perspectives*, *119*(11), 1665-1670. https://doi.org/10.1289/ehp.1103548

Perelló, G., Vicente, E., Castell, V., Llobet, J. M., Nadal, M. y Domingo, J. L. (2015). Dietary intake of trace elements by the population of Catalonia (Spain): results from a total diet study. *Food Additives & Contaminants: Part A*, *32*(5), 1-8. https://doi.org/10.1080/19440049.2015.1018844

Peres, T. V., Schettinger, M. R. C., Chen, P., Carvalho, F., Avila, D. S., Bowman, A. B. y Aschner, M. (2016). «Manganese-induced neurotoxicity: A review of its behavioral consequences and neuroprotective strategies». *BMC Pharmacology and Toxicology*, *17*(1), 57. https://doi.org/10.1186/s40360-016-0099-0

Pesce, G., Sesé, L., Calciano, L., Travert, B., Dessimond, B., Maesano, C. N., Ferrante, G., Huel, G., Prud'homme, J., Guinot, M.,

Soomro, M. H., Baloch, R. M., Lhote, R. y Annesi-Maesano, I. (2021). Foetal exposure to heavy metals and risk of atopic diseases in early childhood. *Pediatric Allergy and Immunology*, *32*(2), 242-250. https://doi.org/10.1111/pai.13397

Peters, B. A., Hall, M. N., Liu, X., Slavkovich, V., Ilievski, V., Alam, S., Siddique, A. B., Islam, T., Graziano, J. H. y Gamble, M. V. (2015). Renal function is associated with indicators of arsenic methylation capacity in Bangladeshi adults. *Environmental Research*, *143*, 123-130. https://doi.org/10.1016/j.envres.2015.10.001

Punshon, T., Davis, M. A., Marsit, C. J., Theiler, S. K., Baker, E. R., Jackson, B. P., Conway, D. C. y Karagas, M. R. (2015). Placental arsenic concentrations in relation to both maternal and infant biomarkers of exposure in a US cohort. *Journal of Exposure Science and Environmental Epidemiology*, *25*, 599-603. https://doi.org/10.1038/jes.2015.16

Punshon, T., Li, Z., Marsit, C. J., Jackson, B. P., Baker, E. R. y Karagas, M. R. (2016). Placental Metal Concentrations in Relation to Maternal and Infant Toenails in a U.S. Cohort. *Environmental Science & Technology*, *50*(3), 1587-1594. https://doi.org/10.1021/acs.est.5b05316

Quansah, R., Armah, F. A., Essumang, D. K., Luginaah, I., Clarke, E., Marfoh, K., Cobbina, S. J., Nketiah-amponsah, E., Namujju, P. B., Obiri, S. y Dzodzomenyo, M. (2015). Association of Arsenic with Adverse Pregnancy Outcomes / Infant Mortality: A Systematic Review and Meta-Analysis. *Environmental Health Perspectives*, *123*, 412-421. https://doi.org/10.1289/ehp.1307894

Rahman, M. M., Hossain, K. F. B., Banik, S., Sikder, M. T., Akter, M., Bondad, S. E. C., Rahaman, M. S., Hosokawa, T., Saito, T. y Kurasaki, M. (2019). Selenium and zinc protections against metal-(loids)-induced toxicity and disease manifestations: A review. *Ecotoxicology and Environmental Safety*, *168*(October 2018), 146-163. https://doi.org/10.1016/j.ecoenv.2018.10.054

Rebelo, F. M. y Caldas, E. D. (2016). Arsenic, lead, mercury and cadmium: Toxicity, levels in breast milk and the risks for breastfed infants. *Environmental Research*, *151*, 671-688. https://doi.org/10.1016/j.envres.2016.08.027

Rivera-Núñez, Z., Ashrap, P., Barrett, E. S., Watkins, D. J., Cathey, A. L., Vélez-Vega, C. M., Rosario, Z., Cordero, J. F., Alshawabkeh, A. y Meeker, J. D. (2021). Association of biomarkers of exposure to metals and metalloids with maternal hormones in pregnant women from Puerto Rico. *Environment International*, *147*. https://doi.org/10.1016/j.envint.2020.106310

Rocha-Amador, D., Navarro, M. E., Carrizales, L., Morales, R. y Calderón, J. (2007). Decreased intelligence in children and exposure to fluoride and arsenic in drinking water. *Cadernos de Saúde Pública*, *23*(suppl 4), S579-S587. https://doi.org/10.1590/S0102-311X2007001600018

Rock, K. D. y Patisaul, H. B. (2018). Environmental Mechanisms of Neurodevelopmental Toxicity. *Current Environmental Health Reports*, *5*(1), 145-157. https://doi.org/10.1007/s40572-018-0185-0.Environmental

Rojas-Rueda, D., Vrijheid, M., Robinson, O., Marit, A. G., Gražulevičienė, R., Slama, R. y Nieuwenhuijsen, M. (2019). Environmental burden of childhood disease in Europe. *International Journal of Environmental Research and Public Health*, *16*(1084). https://doi.org/10.3390/ijerph16061084

Röllin, H. B., Channa, K., Olutola, B. G. y Odland, J. O. (2017). Evaluation of in utero exposure to arsenic in South Africa. *Science of the Total Environment*, *575*, 338-346. https://doi.org/10.1016/j.scitotenv.2016.10.044

Rosado, J. L., Ronquillo, D., Kordas, K., Rojas, O., Alatorre, J., Lopez, P., Garcia-Vargas, G., Caamaño, M. del C., Cebrián, M. E. y Stoltzfus, R. J. (2007). Arsenic exposure and cognitive performance in Mexican Schoolchildren. *Environmental Health Perspectives*, *115*, 1371-1375. https://doi.org/10.1289/ehp.9961

Rose, M., Baxter, M. alcom, Brereton, N. y Baskaran, C. (2010). Dietary exposure to metals and other elements in the 2006 UK total diet study and some trends over the last 30 years. *Food Additives and Contaminants*, *27*(10), 1380-1404. https://doi.org/10.1080/19440049.2010.496794

Ruan, F., Zhang, J., Liu, J., Sun, X., Li, Y., Xu, S. y Xia, W. (2022). Association between prenatal exposure to metal mixtures and early childhood allergic diseases. *Environmental Research*, *206*(December 2021), 112615. https://doi.org/10.1016/j.envres.2021.112615

Rubio, C., Gutiérrez, Á. J., Revert, C., Reguera, J. I., Burgos, A. y Hardisson, A. (2009). Daily dietary intake of iron, copper, zinc and manganese in a Spanish population. *International Journal of Food Sciences and Nutrition*, *60*(7), 590-600. https://doi.org/10.3109/09637480802039822

Ruiz-Azcona, L., Fernández-Olmo, I., Expósito, A., Markiv, B., Paz-Zulueta, M., Parás-Bravo, P., Sarabia-Cobo, C. y Santibáñez,

M. (2021). Impact of environmental airborne manganese exposure on cognitive and motor functions in adults: A systematic review and meta-analysis. *International Journal of Environmental Research and Public Health*, *18*(8). https://doi.org/10.3390/ijerph18084075

Saint-Jacques, N., Parker, L., Brown, P. y Dummer, T. J. (2014). Arsenic in drinking water and urinary tract cancers: A systematic review of 30 years of epidemiological evidence. *Environmental Health*, *13*, 44. https://doi.org/10.1186/1476-069X-13-44

Salinas, R. D., Connolly, D. R. y Song, H. (2020). Epigenetics in Neurodevelopment. *Neuropathology and applied neurobiology*, *46*(1), 6-27. https://doi.org/10.1111/nan.12608

Sanchez, T. R., Perzanowski, M. y Graziano, J. H. (2016). Inorganic arsenic and respiratory health, from early life exposure to sex-specific effects: a systematic review. *Environmental Research*, *147*, 537-555. https://doi.org/10.1016/j.envres.2016.02.009

Santamaria, A. B. y Sulsky, S. I. (2010). Risk assessment of an essential element: Manganese. *Journal of Toxicology and Environmental Health - Part A: Current Issues*, *73*(2), 128-155. https://doi.org/10.1080/15287390903337118

Schlebusch, C. M., Gattepaille, L. M., Engström, K., Vahter, M., Jakobsson, M. y Broberg, K. (2015). Human adaptation to arsenic-rich environments. *Molecular Biology and Evolution*, *32*(6), 1544-1555. https://doi.org/10.1093/molbev/msv046

Schug, T. T., Barouki, R., Gluckman, P. D., Grandjean, P., Hanson, M. y Heindel, J. J. (2013). Environmental Stressors in the Developmental Origins of Disease—Evidence and Mechanisms. *Toxicological Sciences*, *131*(2), 343-350.

Selevan, S. G., Kimmela, C. A. y Mendola, P. (2000). Identifying Critical Windows of Exposure for Children's Health. *Environmental Health Perspectives*, *108*(3), 451-455.

Shaji, E., Santosh, M., Sarath, K. ., Prakash, P., Deepchand, V. y Divya, B. . (2021). Arsenic contamination of groundwater: A global synopsis with focus on the Indian Peninsula. *Geoscience Frontiers*, *12*(3), 101079. https://doi.org/10.1016/j.gsf.2020.08.015

Shen, H., Niu, Q., Xu, M., Rui, D., Xu, S., Feng, G., Ding, YusongLi, S. y Jing, M. (2016). Factors Affecting Arsenic Methylation in Arsenic-Exposed Humans : A Systematic Review and Meta-Analysis. *International Journal of Environmental Research and Public Health*, *13*(205). https://doi.org/10.3390/ijerph13020205

Shih, Y. H., Chen, H. Y., Christensen, K., Handler, A., Turyk, M. E. y Argos, M. (2021). Prenatal exposure to multiple metals and birth outcomes: An observational study within the National Children's Study cohort. *Environment International*, *147*, 106373. https://doi.org/10.1016/j.envint.2020.106373

Siddiqui, S., Chatterjee, U., Kumar, D., Siddiqui, A. y Goyal, N. (2008). Neuropsychology of prefrontal cortex. *Indian Journal of Psychiatry*, *50*(3), 202-208. https://doi.org/10.4103/0019-5545.43634

Signes-Pastor, A. J., Carey, M., Carbonell-Barrachina, A. A., Moreno-Jiménez, E., Green, A. J. y Meharg, A. A. (2016). Geographical variation in inorganic arsenic in paddy field samples and commercial rice from the Iberian Peninsula. *Food Chemistry*, *202*, 356-363. https://doi.org/10.1016/j.foodchem.2016.01.117

Signes-Pastor, A. J., Desai, G., García-Villarino, M., Karagas, M. R. y Kordas, K. (2021). Exposure to a Mixture of Metals and Growth Indicators in 6–11-Year-Old Children from the 2013–2016 NHANES. *Exposure and Health*, *13*(2), 173-184. https://doi.org/10.1007/s12403-020-00371-8

Signes-Pastor, A. J., Martinez-Camblor, P., Baker, E., Madan, J., Guill, M. F. y Karagas, M. R. (2021). Prenatal exposure to arsenic and lung function in children from the New Hampshire Birth Cohort Study. *Environment International*, *155*, 106673. https://doi.org/10.1016/j.envint.2021.106673

Signes-Pastor, A. J., Vioque, J., Navarrete-Muñoz, E. M., Carey, M., García-Villarino, M., Fernández-Somoano, A., Tardón, A., Santa-Marina, L., Irizar, A., Casas, M., Guxens, M., Llop, S., Soler-Blasco, R., García-de-la-hera, M., Karagas, M. R. y Meharg, A. A. (2019). Inorganic arsenic exposure and neuropsychological development of children of 4–5 years of age living in Spain. *Environmental Research*, *174*, 135-142. https://doi.org/10.1016/j.envres.2019.04.028

Skogheim, T. S., Weyde, K. V. F., Engel, S. M., Aase, H., Surén, P., Øie, M. G., Biele, G., Reichborn-Kjennerud, T., Caspersen, I. H., Hornig, M., Haug, L. S. y Villanger, G. D. (2021). Metal and essential element concentrations during pregnancy and associations with autism spectrum disorder and attention-deficit/hyperactivity disorder in children. *Environment International*, *152*(February). https://doi.org/10.1016/j.envint.2021.106468

Spencer, A. (1999). Whole Blood Manganese Levels in Pregnancy and the Neonate. *Nutrition*, *15*(10), 731-734.

Spratlen, M. J., Gamble, M. V, Grau-Perez, Ma., Chin-Chi, K., Best, L. G., Yracheta, J., Francesconi, K., Goessler, W., Mossavar-Rahmani, Y., Hall, M., Umans, J. G., Fretts, A. y Navas-Acien, A. (2017). Arsenic metabolism and one-carbon metabolism at low- moderate arsenic exposure: evidence from the Strong Heart Study. *Food and chemical toxicology*, *105*, 387-397. https://doi.org/10.1016/j.fct.2017.05.004.Arsenic

Stajnko, A., Šlejkovec, Z., Mazej, D., France-štiglic, A., Sešek, A., Prpić, I., Špirić, Z., Horvat, M. y Falnoga, I. (2019). Arsenic metabolites; selenium; and AS3MT, MTHFR, AQP4, AQP9, SELENOP, INMT, and MT2A polymorphisms in Croatian-Slovenian population from PHIME-CROME study. *Environmental Research*, *170*(October 2018), 301-319. https://doi.org/10.1016/j.envres.2018.11.045

Stiles, J. y Jernigan, T. L. (2010). *The Basics of Brain Development*. 327-348. https://doi.org/10.1007/s11065-010-9148-4

Styblo, M., Del Razo, L. M., Vega, L., Germolec, D. R., LeCluyse, E. L., Hamilton, G. A., Reed, W., Wang, C., Cullen, W. R. y Thomas, D. J. (2000). Comparative toxicity of trivalent and pentavalent inorganic and methylated arsenicals in rat and human cells. *Archives of Toxicology*, *74*(6), 289-299. https://doi.org/10.1007/s002040000134

Sun, H. J., Xiang, P., Luo, J., Hong, H., Lin, H., Li, H. B. y Ma, L. Q. (2016). Mechanisms of arsenic disruption on gonadal, adrenal and thyroid endocrine systems in humans: A review. *Environment International*, *95*, 61-68. https://doi.org/10.1016/j.envint.2016.07.020

Sun, H., Rathinasabapathi, B., Wu, B., Luo, J., Pu, L. y Ma, L. Q. (2014). Arsenic and selenium toxicity and their interactive effects in humans. *Environment International*, *69*, 148-158. https://doi.org/10.1016/j.envint.2014.04.019

Takser, L., Lafond, J., Bouchard, M., St-amour, G. y Mergler, D. (2004). Manganese levels during pregnancy and at birth : relation to environmental factors and smoking in a Southwest Quebec population. *Environmental Research*, *95*, 119-125. https://doi.org/10.1016/j.envres.2003.11.002

Takser, L., Mergler, D., Hellier, G., Sahuquillo, J. y Huel, G. (2003). Manganese, monoamine metabolite levels at birth, and child psychomotor development. *NeuroToxicology*, *24*(4-5), 667-674. https://doi.org/10.1016/S0161-813X(03)00058-5

Taylor, V. F., Li, Z., Sayarath, V., Palys, T. J., Morse, K. R., Scholz-Bright, R. A. y Karagas, M. R. (2017). Distinct arsenic metabolites following seaweed consumption in humans. *Scientific Reports*, *7*(1), 1-9. https://doi.org/10.1038/s41598-017-03883-7

Taylor, V., Goodale, B., Raab, A., Schwerdtle, T., Reimer, K., Conklin, S., Karagas, M. R. y Francesconi, K. A. (2017). Human Exposure to Organic Arsenic Species from Seafood. *Science of The Total Environment*, *15*(580), 266-282. https://doi.org/10.1016/j.scitotenv.2016.12.113.Human

Tsai, T. L., Wang, S. L., Hsieh, C. J., Wen, H. J., Kuo, C. C., Liu, H. J., Sun, C. W., Chen, M. L. y Wu, M. T. (2021). Association between Prenatal Exposure to Metals and Atopic Dermatitis among Children Aged 4 Years in Taiwan. *JAMA Network Open*, *4*(10), 1-14. https://doi.org/10.1001/jamanetworkopen.2021.31327

Tseng, C.-H. (2008). A review on environmental factors regulating arsenic methylation in humans. *Toxicology and Applied Pharmacology*, *235*, 338-350. https://doi.org/10.1016/j.taap.2008.12.016

Tsuji, J. S., Perez, V., Garry, M. R. y Alexander, D. D. (2014). Association of low-level arsenic exposure in drinking water with cardiovascular disease: A systematic review and risk assessment. *Toxicology*, *323*, 78-94. https://doi.org/10.1016/j.tox.2014.06.008

Tuschl, K., Mills, P. B. y Clayton, P. T. (2013). Manganese and the Brain. En K. P. Bhatia & S. A. Schneider (Eds.), *Metal Related Neurodegenerative Disease* (1.ª ed., Vol. 110, pp. 277-312). Elsevier Inc. https://doi.org/10.1016/B978-0-12-410502-7.00013-2

U.S. Department of Agriculture: Agricultural Research Service USDA. (2007). *National Nutrient Database for Standard Reference, Release 25.* https://www.ars.usda.gov/ARSUserFiles/80400525/Data/SR25/sr25_doc.pdf

U.S. Environmental Protection Agency. (2008). *Child-Specific Exposure Factors Handbook*.

U.S. Food and Drug Administration. (2017). *Total Diet Study Elements Results Summary Statistics Market Baskets 2006 through 2013* (Número February).

U.S Food and Drug Administration. (2016). *Arsenic in Rice and Rice Products Risk Assessment Report*.

Universitat de València. (2012). *Guía de uso para un lenguaje igualitario (castellano)* (p. 163).

Upadhyay, M. K., Shukla, A., Yadav, P. y Srivastava, S. (2019). A review of arsenic in crops , vegetables, animals and food products. *Food Chemistry*, *276*, 608-618. https://doi.org/10.1016/j.foodchem.2018.10.069

Vahter, M. (1999). Methylation of inorganic arsenic in different mammalian species and population groups. *Science progress*, *82*(1), 69-88. https://doi.org/10.1177/003685049908200104

Vahter, M. (2002). Mechanisms of arsenic biotransformation. *Toxicology*, *181-182*, 211-217. https://doi.org/10.1016/S0300-483X(02)00285-8

Vahter, M. (2009). Effects of Arsenic on Maternal and Fetal Health. *Annual Review of Nutrition*, *29*, 381-399. https://doi.org/10.1146/annurev-nutr-080508-141102

Vahter, M., Concha, G., Nermell, B., Nilsson, R., Dulout, F. y Natarajan, A. T. (1995). A unique metabolism of inorganic arsenic in native Andean women. *European journal of pharmacology. Environmental toxicology and pharmacology section*, *293*(4), 455-462. https://doi.org/10.1016/0926-6917(95)90066-7

Vahter, M. E. (2007). Interactions between Arsenic-Induced Toxicity and Nutrition in Early Life. *The Journal of Nutrition*, *137*(12), 2798-2804. https://doi.org/10.1093/jn/137.12.2798

Vahter, M., Skröder, H., Rahman, S. M., Levi, M., Derakhshani Hamadani, J. y Kippler, M. (2020). Prenatal and childhood arsenic exposure through drinking water and food and cognitive abilities at 10 years of age: A prospective cohort study. *Environment International*, *139*, 105723. https://doi.org/10.1016/j.envint.2020.105723

Valeri, L., Mazumdar, M. M., Bobb, J. F., Henn, B. C., Rodrigues, E., Sharif, O. I. A., Kile, M. L., Quamruzzaman, Q., Afroz, S., Golam, M., Amarasiriwardena, C., Bellinger, D. C., Christiani, D. C., Coull, B. A. y Wright, R. O. (2017). The Joint Effect of Prenatal Exposure to Metal Mixtures on Neurodevelopmental Outcomes at 20 – 40 Months of Age : Evidence from Rural Bangladesh. *Environmental Health Perspectives*, *125*(6), 067015. https://doi.org/10.1289/EHP614

Vaughan Watson, C., Lewin, M., Ragin-wilson, A., Jones, R., Jarrett, J. M., Wallon, K., Ward, C., Hilliard, N., Irvin-barnwell, E., Watson, V., Christina, Lewin, M., Ragin-wilson, A., Jones, R., Jarrett, M., Wallon, K., Ward, C., Hilliard, N. y Irvin-barnwell, E. (2020). Characterization of trace elements exposure in pregnant women in the United States, NHANES 1999–2016. *Environmental Research*, *183*, 109208. https://doi.org/10.1016/j.envres.2020.109208

Vineis, P., Chatziioannou, A., Cunliffe, V. T., Flanagan, J. M., Hanson, M., Kirsch-Volders, M. y Kyrtopoulos, S. (2017). Epigenetic memory in response to environmental stressors. *FASEB Journal*, *31*(6), 2241-2251. https://doi.org/10.1096/fj.201601059RR

Vioque, J., Navarrete-Muñoz, E.-M., Gimenez-Monzó, D., García-de-la-Hera, M., Granado, F., Young, I. S., Ramón, R., Ballester, F., Murcia, M., Rebagliato, M. y Iñiguez, C. (2013). Reproducibility and validity of a food frequency questionnaire among pregnant women in a Mediterranean area. *Nutrition Journal*, *12*, 26. https://doi.org/10.1186/1475-2891-12-26

Von Ehrenstein, O. S., Poddar, S., Yuan, Y., Mazumder, D. G., Eskenazi, B., Basu, A., Hira-Smith, M., Ghosh, N., Lahiri, S., Haque, R., Ghosh, A., Kalman, D., Das, S. y Smith, A. H. (2007). Children's intellectual function in relation to arsenic exposure. *Epidemiology*, *18*(1), 44-51. https://doi.org/10.1097/01.ede.0000248900.65613.a9

Wang, F., Zou, Y., Shen, Y., Zhong, Y., Lv, Y., Huang, D., Chen, K., Li, Q., Qing, L., Xia, B., Su, C., Ma, S. y Yang, X. (2015). Synergistic impaired effect between smoking and manganese dust exposure on pulmonary ventilation function in guangxi manganese-exposed workers healthy cohort (GXMEWHC). *PLoS ONE*, *10*(2), e011655. https://doi.org/10.1371/journal.pone.0116558

Wang, G., Tang, W. Y., Wills-Karp, M., Ji, H., Bartell, T. R., Ji, Y., Hong, X., Pearson, C., Cheng, T. L. y Wang, X. (2021). A Nonlinear Relation between Maternal Red Blood Cell Manganese Concentrations and Child Blood Pressure at Age 6-12 y: A Prospective Birth Cohort Study. *Journal of Nutrition*, *151*(3), 570-578. https://doi.org/10.1093/jn/nxaa368

Wang, X., Wu, Y., Sun, X., Guo, Q., Xia, W., Wu, Y., Li, J., Xu, S. y Li, Y. (2021). Arsenic exposure and metabolism in relation to blood pressure changes in pregnant women. *Ecotoxicology and Environmental Safety*, *222*, 112527. https://doi.org/10.1016/j.ecoenv.2021.112527

Wasserman, G. A., Liu, X., LoIacono, N. J., Kline, J., Factor-Litvak, P., Van Geen, A., Mey, J. L., Levy, D., Abramson, R., Schwartz, A. y Graziano, J. H. (2014). A cross-sectional study of well water arsenic and child IQ in Maine schoolchildren. *Environmental Health*, *13*(23). https://doi.org/10.1186/1476-069X-13-23

Wasserman, G. A., Liu, X., Parvez, F., Chen, Y., Factor-Litvak, P., LoIacono, N. J., Levy, D., Shahriar, H., Uddin, M. N., Islam, T., Lomax, A., Saxena, R., Gibson, E. A., Kioumourtzoglou, M. A., Balac, O., Sanchez, T., Kline, J. K., Santiago, D., Ellis, T., … Graziano, J. H. (2018). A cross-sectional study of water arsenic exposure and intellectual function in adolescence in Araihazar, Bangladesh. *Environment International*, *118*(June), 304-313. https://doi.org/10.1016/j.envint.2018.05.037

Wasserman, G. A., Liu, X., Parvez, F., Factor-Litvak, P., Habibul, A., Levy, D., Kline, J., Van Geen, A., Mey, J., Slavkovich, V., Siddique, A. B., Islam, T. y Graziano, J. H. (2011). Arsenic and manganese exposure and children's intellectual function. *Neurotoxicology*, *32*(4), 450-457. https://doi.org/10.1109/TMI.2012.2196707.Separate

Wasserman, G. A., Liu, X., Parvez, F., Factor-Litvak, P., Kline, J., Siddique, A. B., Shahriar, H., Uddin, M. N., Van Geen, A., Mey, J. L., Balac, O. y Graziano, J. H. (2016). Child intelligence and reductions in water arsenic and manganese: A two-year follow-up study in Bangladesh. *Environmental Health Perspectives*, *124*(7), 1114-1120. https://doi.org/10.1289/ehp.1509974

Williams, P. N., Villada, A., Deacon, C., Raab, A., Figuerola, J., Green, A. J., Feldmann, J. y Meharg, A. A. (2007). Greatly enhanced arsenic shoot assimilation in rice leads to elevated grain levels compared to wheat and barley. *Environmental science & technology*, *41*(19), 6854-6859.

World Health Organization. (2000a). *Air Quality Guidelines for Europe* (Second edi). World Health Organization.

World Health Organization. (2000b). Arsenic. En *WHO air quality guidelines for Europe, 2nd edition (CD ROM version)*. WHO Regional Office for Europe.

World Health Organization. (2001). *Environmental Health Criteria 224. Arsenic and Arsenic Compounds*. World Health Organization, International Programme on Chemical Safety. https://doi.org/NLM Classification: QV 294

World Health Organization. (2005). *Children ' s health and environment. Developing action plans*. World Health Organization.

World Health Organization. (2011). *Manganese in Drinking-water*. World Health Organization.

World Health Organization. (2012). Developmental difficulties in early childhood: Prevention, early identification, assessment and intervention in low- and middle-income countries. A review. En *World Health Organization*. World Health Organization.

World Health Organization. (2017). *Guidelines for drinking-water quality: fourth edition*. World Health Organization.

Wright, R. O., Amarasiriwardena, C., Woolf, A. D., Jim, R. y Bellinger, D. C. (2006). Neuropsychological correlates of hair arsenic, manganese, and cadmium levels in school-age children residing near a hazardous waste site. *NeuroToxicology*, *27*(2), 210-216. https://doi.org/10.1016/j.neuro.2005.10.001

Xu, L., Mondal, D. y Polya, D. A. (2020). Positive association of cardiovascular disease (CVD) with chronic exposure to drinking water arsenic (As) at concentrations below the WHO provisional guideline value: A systematic review and meta-analysis. *International Journal of Environmental Research and Public Health*, *17*, 2536. https://doi.org/10.3390/ijerph17238947

Xu, X.-Y., McGrath, S. P., Meharg, A. A. y Zhao, F.-J. (2008). Growing rice aerobically markedly decreases arsenic accumulation. *Environmental Science and Technology*, *42*(15), 5574-5579. https://doi.org/10.1021/es800324u

Yamamoto, M., Sakurai, K., Eguchi, A., Yamazaki, S., Nakayama, S. F., Isobe, T., Takeuchi, A., Sato, T., Hata, A., Mori, C., Nitta, H., Ohya, Y., Kishi, R., Yaegashi, N., Hashimoto, K., Ito, S., Yamagata, Z., Inadera, H., Kamijima, M., … Katoh, T. (2019). Association between blood manganese level during pregnancy and birth size: The Japan environment and children's study (JECS). *Environmental Research*, *172*(February), 117-126. https://doi.org/10.1016/j.envres.2019.02.007

Yu, H., Wu, B., Zhang, X. X., Liu, S., Yu, J., Cheng, S., Ren, H. Q. y Ye, L. (2016). Arsenic Metabolism and Toxicity Influenced by Ferric Iron in Simulated Gastrointestinal Tract and the Roles of Gut Microbiota. *Environmental Science and Technology*, *50*(13), 7189-7197. https://doi.org/10.1021/acs.est.6b01533

Yu, X., Cao, L. y Yu, X. (2013). Elevated cord serum manganese level is associated with a neonatal high ponderal index. *Environmental Research*, *121*, 79-83. https://doi.org/10.1016/j.envres.2012.11.002

Yusà, V., Dualde, P., Pardo, O., Coscollà, C., López, A. y Fernández, S. (2020). *Evaluación de la exposición a contaminantes alimentarios y ambientales mediante biomonitorización humana en la Comunitat Valenciana*. Generalitat Valenciana.

Zablotsky, B., Black, L. I. y Blumberg, S. J. (2017). Estimated Prevalence of Children With Diagnosed Developmental Disabilities

in the United States, 2014-2016. *National Center for Health Statistics data brief*, *291*, 1-8.

Zhang, Q., Zhang, X., Li, S., Liu, H., Liu, L., Huang, Q., Hou, Y., Liang, X., Cui, B., Zhang, M., Xia, L., Zhang, L., Li, C., Li, J., Sun, G. y Tang, N. (2021). Joint effect of urinary arsenic species and serum one-carbon metabolism nutrients on gestational diabetes mellitus: A cross-sectional study of Chinese pregnant women. *Environment International*, *156*, 106741. https://doi.org/10.1016/j.envint.2021.106741

Zhao, J., Li, A., Mei, Y., Zhou, Q., Li, Y., Li, K. y Xu, Q. (2021). The association of arsenic exposure with hypertension and blood pressure: A systematic review and dose–response meta-analysis. *Environmental Pollution*, *289*, 117914. https://doi.org/10.1016/j.envpol.2021.117914

Zhao, Y., Li, M., Tian, X., Xie, J., Liu, P., Ying, X., Wang, M., Yuan, J., Gao, Y., Tian, F. y Yan, X. (2021). Effects of arsenic exposure on lipid metabolism: a systematic review and meta-analysis. *Toxicology Mechanisms and Methods*, *31*(3), 1-12. https://doi.org/10.1080/15376516.2020.1864537

Zheng, T., Zhang, J., Sommer, K., Bassig, B. A., Zhang, X., Braun, J., Xu, S., Boyle, P., Zhang, B., Shi, K., Buka, S., Liu, S., Li, Y., Qian, Z., Dai, M., Romano, M., Zou, A. y Kelsey, K. (2016). Effects of Environmental Exposures on Fetal and Childhood Growth Trajectories. En *Annals of Global Health* (Vol. 82, Número 1, pp. 41-99). https://doi.org/10.1016/j.aogh.2016.01.008

Zhong, Q., Cui, Y., Wu, H., Niu, Q., Lu, X., Wang, L. y Huang, F. (2019). Association of maternal arsenic exposure with birth size: A systematic review and meta-analysis. *Environmental Toxicology and Pharmacology*, *69*, 129-136. https://doi.org/10.1016/j.etap.2019.04.007

Zhou, Z., Chen, G., Li, P., Rao, J., Wang, L., Yu, D., Lin, D., Fan, D., Ye, S., Wu, S., Gou, X., Wang, H., Guo, X., Lin, L., Suo, D. y Liu, Z. (2021). Prospective association of metal levels with gestational diabetes mellitus and glucose: A retrospective cohort study from South China. *Ecotoxicology and Environmental Safety*, *210*, 111854. https://doi.org/10.1016/j.ecoenv.2020.111854

ANEXOS

ANEXO 1:

Material suplementario de los artículos

Supplemental Material

Prenatal manganese exposure and effects on neuropsychological development in early childhood in the INMA cohort.

Authors: Raquel Soler-Blasco, Mario Murcia, Manuel Lozano, Llúcia González-Safont, Rubén Amorós, Jesús Ibarluzea, Karin Broberg, Amaia Irizar, Maria-José Lopez-Espinosa, Nerea Lertxundi, Loreto Santa Marina, Ferran Ballester, Sabrina Llop.

Table S1. Differences between included and non-included population in the study. INMA Project (Valencia and Gipuzkoa, Spain, 2004-2008).

Table S2: Multivariate linear regression analysis between maternal Mn levels and child neuropsychological development assessed by Bayley Scores at 1 year old stratified by child sex and maternal ferritin status. INMA Project, Spain

Table S3. Sensitivity analysis. Multivariate linear regression analysis between maternal Mn levels and child neuropsychological development assessed by Bayley Scores at 1 year old, excluding certain population subgroups. INMA Project, Spain

Figure S1: Association between maternal serum Mn at first trimester of pregnancy and the mental and psychomotor scales assessed by the Bayley Scores of Infant Development at 12 months of age stratified by child's sex and maternal ferritin status.

Table S1: Differences between included and non-included population in the study. INMA Project (Valencia and Gipuzkoa, Spain, 2004-2008).

	Included Population (n= 1179)	Non-included Population (n=220)	
	N[1] (%)	N[1] (%)	p[2]
Area of study			
Gipuzkoa	534 (45)	78 (35)	<0.01
Valencia	645 (55)	142 (65)	
Gestational age at sampling	13.0 (1.2)[4]	13.8 (1.3)	<0.01[3]
Maternal age (years)			
<25	77 (7)	26 (12)	<0.01
25-29	382 (32)	84 (38)	
30-34	527 (45)	72 (33)	
≥35	193 (16)	38 (17)	
Maternal country of birth			
Spain	1082 (92)	199 (90)	0.61
Others	97 (8)	21 (10)	
BMI before pregnancy (Kg/m^2)			
Healthy (18.5–<25)	844 (72)	154 (70)	0.15
Low weight (<18.5)	45 (4)	14 (6)	
Overweight (25–<30)	207 (18)	31 (14)	
Obese (>30)	83 (7)	20 (9)	
Parity			
0	656 (56)	108 (49)	0.09
≥1	523 (44)	112 (51)	
Parental social class			
I+II (high)	393 (33)	47 (21)	<0.01
III	293 (25)	54 (25)	
IV+V (low)	493 (42)	119 (54)	
Maternal educational level			
Up to primary	272 (23)	76 (44)	<0.01
Secondary	471 (40)	81 (41)	
University	434 (37)	63 (15)	
Area of residence			
Urban/Metropolitan	370 (31)	55 (25)	0.07
Semi-urban/Rural	809 (69)	165 (75)	
Maternal smoking habit until 12 wg			
No	942 (81)	159 (77)	0.21
Yes	223 (19)	48 (23)	
Maternal ferritin serum at 12 wg (µg/L)			
≥15 mg/L	209 (19)	36 (19)	0.87
<15 mg/L	870 (81)	158 (81)	

[1] Missing values for some variables not included in percentages: Gestational age at sampling in non-included population (145), maternal education level (2 in included population), maternal smoking habit until 12 wg (3 in non-included population, 14 in included population), maternal ferritin serum at 12 wg (26 in non-included population, 100 in included population).

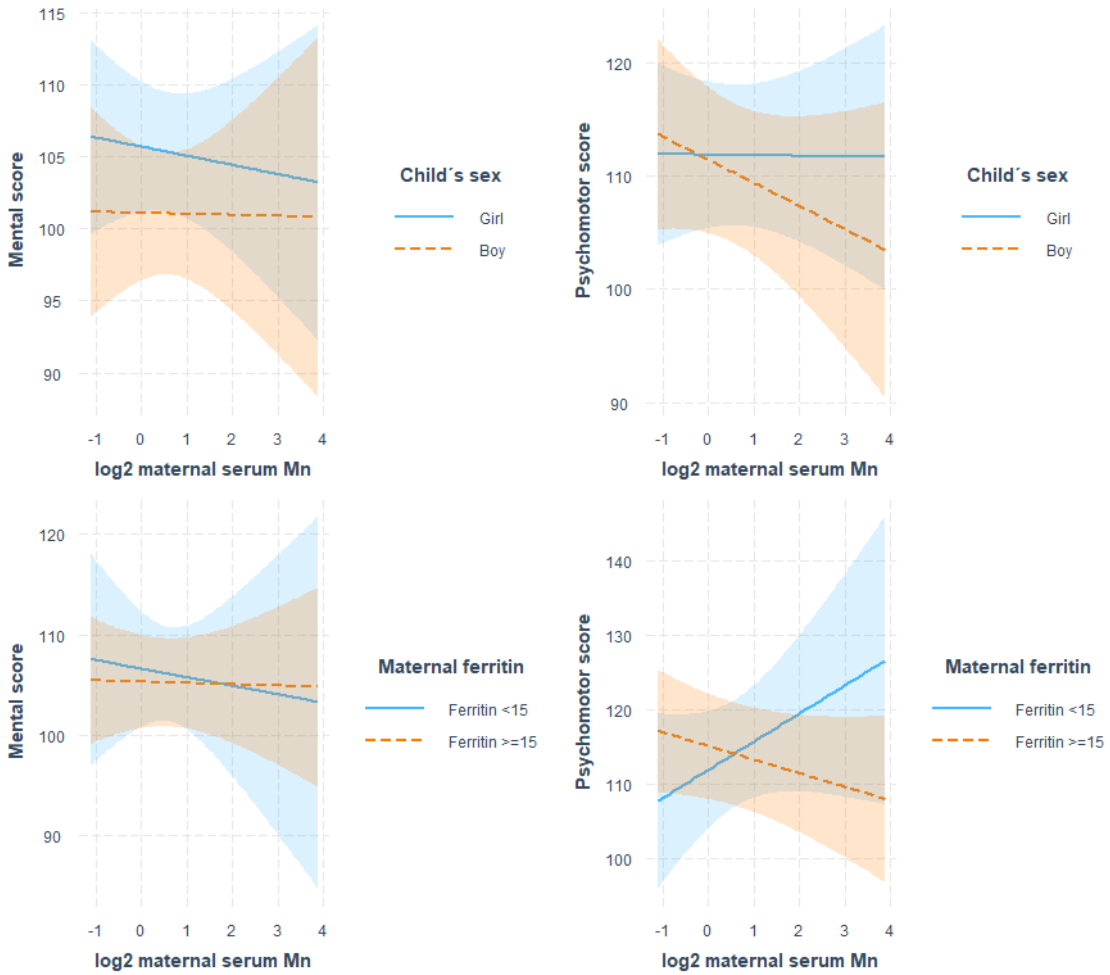
[2] p-value from Chi-squared test; [3] p-value from t-test; [4] Mean (standard deviation); N: sample size; BMI: Body mass index; wg: weeks of gestation.

Table S2: Multivariate linear regression analysis between maternal Mn levels and child neuropsychological development assessed by Bayley Scores at 1 year old stratified by child sex and maternal ferritin status. INMA Project, Spain

		Mental scale				Psychomotor scale			
		beta	95%CI		P interaction	beta	95%CI		P interaction
Child's Sex	Boys	-0.06	-3.71	3.58	0.81	-2.05	-5.44	1.34	0.39
	Girls	-0.53	-3.54	2.49		-0.05	-3.13	3.02	
Maternal ferritin status	Ferritin<15	-1.16	-6.91	4.60	0.76	-1.83	-4.45	0.80	0.08
	Ferritin≥15	-0.20	-2.85	2.46		3.48	-2.38	9.34	

Maternal serum Mn concentrations were log2 transformed.
All models adjusted for children's age at evaluation, psychologist, area of study (Gipuzkoa/ Valencia), child's sex (except models stratified by child's sex) and consumption of nuts at first trimester of pregnancy.
Mental scale model additionally adjusted for maternal age and body mass index before pregnancy.
Psychomotor scale model additionally adjusted for parental social class, maternal educational level and paternal age.

Figure S1: Association between maternal serum Mn at first trimester of pregnancy and the mental and psychomotor scales assessed by the Bayley Scores of Infant Development at 12 months of age stratified by child's sex and maternal ferritin status.



Both models adjusted for children's age at evaluation, psychologist, area of study, child's sex (except models stratified by child's sex) and consumption of nuts at first trimester of pregnancy. Mental scale model additionally adjusted for maternal age and body mass index before pregnancy. Psychomotor scale model additionally adjusted for parental social class, maternal educational level and paternal age.

Table S3: Sensitivity analysis. Multivariate linear regression analysis between maternal Mn levels and child neuropsychological development assessed by Bayley Scores at 1 year old, excluding certain population subgroups. INMA Project, Spain

	Mental scale				Psychomotor scale			
	beta	95%CI		P[1]	beta	95%CI		P[1]
Model 1	-0.39	-2.73	1.95	0.74	-0.92	-3.48	1.65	0.43
Excluding preterm birth (n=48)	-0.33	-2.65	1.99	0.78	-0.65	-2.95	1.66	0.58
Excluding low birth weight (n=55)	-0.55	-2.87	1.77	0.64	-0.77	-3.06	1.52	0.51
Excluding children with an underlying pathology (n=16)	-0.41	-2.74	1.91	0.73	-0.44	-2.66	1.78	0.70
Excluding uncertain quality of test (n=85)	-0.27	-2.59	2.05	0.82	-0.77	-3.10	1.56	0.52
Excluding extreme outlier (n=1)	-0.73	-3.12	1.66	0.55	-1.52	-3.85	0.81	0.20
Model 2	-0.04	-2.83	2.01	0.74	-0.96	-3.72	1.80	0.43
Excluding preterm birth (n=48)	-0.29	-2.71	2.13	0.81	-0.69	-3.14	1.76	0.58
Excluding low birth weight (n=55)	-0.46	-2.88	1.96	0.71	-0.76	-3.20	1.67	0.54
Excluding children with an underlying pathology (n=16)	-0.35	-2.76	2.06	0.78	-0.33	-2.70	2.03	0.78
Excluding uncertain quality of test (n=85)	-0.48	-2.90	1.95	0.70	-0.92	-3.40	1.55	0.46
Excluding extreme outlier (n=1)	-0.78	-3.26	1.71	0.54	-1.60	-4.08	0.87	0.20
Model 3	-0.30	-2.64	2.04	0.80	-0.07	-0.17	0.03	0.51
Excluding preterm birth (n=48)	-0.25	-2.58	2.08	0.83	-0.48	-2.79	1.83	0.68
Excluding low birth weight (n=55)	-0.46	-2.79	1.87	0.70	-0.61	-2.91	1.69	0.60
Excluding children with an underlying pathology (n=16)	-0.31	-2.64	2.02	0.79	-0.25	-2.48	1.99	0.83
Excluding uncertain quality of test (n=85)	-0.22	-2.55	2.11	0.85	-0.64	-2.98	1.70	0.59
Excluding extreme outlier (n=1)	-0.64	-3.04	1.76	0.60	-1.37	-3.71	0.97	0.25

Maternal serum Mn concentrations were log2 transformed.
Model 1: All models adjusted for children's age at evaluation, psychologist, area of study, child's sex and consumption of nuts at first trimester of pregnancy.
Mental scale model additionally adjusted for maternal age and body mass index before pregnancy.
Psychomotor scale model additionally adjusted for parental social class, maternal educational level and paternal age.
Model 2: model 1 + maternal serum ferritin (log2 transformed) at first trimester of pregnancy.
Model 3: model 1 + maternal serum selenium at first trimester of pregnancy.
[1]p-value from ANOVA F- test

Supplemental Material

Title: Urinary arsenic species and methylation efficiency during pregnancy: concentrations and associated factors in Spanish pregnant women.

Authors: Raquel Soler-Blasco, Mario Murcia, Manuel Lozano, Blanca Sarzo, Ana Esplugues, Jesús Vioque , Nerea Lertxundi, Loreto Santa Marina, Aitana Lertxundi, Amaia Irizar, Simone Braeuer , Walter Gossler, Ferran Ballester, Sabrina Llop.

Table S1: Socio-demographic and environmental characteristics of study participants, and differences between included and excluded population in the study. INMA Project (Valencia and Gipuzkoa, Spain, 2003-2008).

Figure S1 Correlations between energy adjusted dietary variables (grams per day). INMA Project (Valencia and Gipuzkoa, Spain, 2003-2008).

Figure S2 Correlations between energy adjusted log2-transformed estimated nutrients intake. INMA Project (Valencia and Gipuzkoa, Spain, 2003-2008).

Figure S3 Correlation between log2-transfomed measured and calibrated[a] As concentrations (µg/g creatinine) . INMA Project (Valencia and Gipuzkoa, Spain, 2003-2008).

Table S2 Geometric mean (95% confident intervals) of total As, total inorganic As, arsenobetaine and As metabolites concentrations of adjusted by creatinine by characteristics of participants. INMA Project (Valencia and Gipuzkoa. Spain. 2003-2008).

Figure S4 Fish and meat sub-categories associated with maternal urinary As, arsenobetaine and As metabolites concentrations

Table S3: Bivariate linear regression between methylation efficiency (measured by calibrated[1] percentage of As metabolites in maternal urine and principal component 1 and 2 of PCA) and estimated nutrients intake (adjusted by calories) and essential and toxics elements factors. INMA Project (Valencia and Gipuzkoa, Spain, 2003-2008).

Table S4 Dietary intake variables (energy adjusted) during the first trimester of pregnancy by study area. INMA Project (Valencia and Gipuzkoa, Spain, 2003-2008).

Table S1: Socio-demographic and environmental characteristics of study participants, and differences between included and excluded population in the study. INMA Project (Valencia and Gipuzkoa, Spain, 2003-2008).

	Included Population (n= 1017)[a]	Excluded-Population (n=448)	
	N[a] (%)	N[a] (%)	p[b]
Area of study			
Gipuzkoa	417 (41.0)	221 (49)	<0.01
Valencia	600 (59.0)	227 (51)	
Gestational age at sampling	12.7 (12.3, 13.4)[c]	13.0 (12.3, 13.7)[c]	0.03
Age (years)			
<25	67 (7)	49 (11)	0.04
25-29	336 (33)	148 (33)	
30-34	443 (44)	181 (40)	
≥35	171 (17)	70 (16)	
Country of birth			
Spain	940 (92)	398 (89)	0.02
Others	77 (8)	50 (11)	
BMI before pregnancy (Kg/m^2)			
<25	772 (76)	329 (74)	0.59
25- <30 (Overweight)	169 (17)	84 (19)	
≥ 30 (Obesity)	76 (7)	33 (7)	
Parity			
0	557 (55)	246 (55)	1.00
≥1	460(45)	202 (45)	
Parental social class			
I+II (high)	345 (34)	113 (25)	<0.01
III	263 (26)	92 (21)	
IV+V (low)	409 (40)	243 (54)	
Educational level			
Up to primary	229 (23)	145 (32)	<0.001
Secondary	407 (40)	176 (39)	
University	379 (37)	127 (28)	
Area of residence			
Non- rural	958 (94)	409 (93)	0.16
Rural	56 (6)	33 (7)	
Proximity to agricultural area			
No	615 (61)	243 (64)	0.62
Yes	391 (39)	139 (36)	
Proximity to industrial area			
No	673 (67)	226 (70)	0.36
Yes	333 (33)	116 (30)	
Tobacco consumption			
No	820 (82)	292 (77)	0.05
Yes	185 (18)	88 (23)	
Alcohol consumption			
No	876 (87)	363 (83)	0.08
Yes	135 (13)	74 (17)	
Rice consumption			
< 1 serving at week	495 (49)	230 (53)	0.54
≥ 1 serving at week	516 (51)	207 (47)	
Fish consumption (grams/day)	63.1 (42.3, 87.4)[c]	57.1 (39.3, 79.6)[c]	<0.01
Vitamins supplements intake			
Yes	975 (96)	417 (93)	0.05
No	42 (4)	31 (7)	

Vitamin B_6 intake[d]			
≤ 1.8 mg/ day	227 (22)	112 (26)	0.20
> 1.8 mg/ day	784 (78)	324 (74)	
Vitamin B_{12} intake[d]			
≤ 4.5 mg/ day	23 (2)	10(2)	1.00
> 4.5 mg/ day	988 (98)	426 (98)	
Folate and acid folic intake[d]			
≤ 600 µg/day	163 (16)	76 (17)	0.53
> 600 µg/day	848 (84)	360 (83)	
Zinc intake[d]			
≤1.6 mg/day	0 (0)	0 (0)	
>1.6 mg/day	1011 (100)	436 (100)	
Iron intake[d]			
≤16 mg/day	49 (5)	23 (5)	0.80
>16 mg/day	961 (95)	413 (95)	
Maternal ferritin serum			
≤15 mg/L	159 (17)		0.007
>15 mg/L	758 (83)		

Note: N, sample size; BMI, Body mass index.

[a] Missing values for some variables not included in percentages: Gestational age (8), BMI before pregnancy, Maternal education level (2), Proximity to industrial area (11), Proximity to agricultural area (11), Maternal smoking habit until 12 weeks of gestation (12), Maternal alcohol consumption until 12 weeks of gestation (6), Maternal rice consumption at 12 weeks of gestation (6), Maternal fish consumption at 12 weeks of gestation (6), Maternal vitamin B_6, B_{12}, Zn, folate and iron intake (6).

[b] p-value, comparing women characteristics between included and excluded population using Fisher's Exact Test for categorical variables and Kruskall Wallis Test for continuous variables.

[c] median and 25^{th}, 75^{th} percentile.

[d] Levels above and below the Population Reference Intake for Fe, Zn and vitamin B_6 and adequate intake for folate and vitamin B_{12} for pregnant women established by the European Food Safety Authority.

Figure S1 Correlations between energy adjusted dietary variables (grams per day). INMA Project (Valencia and Gipuzkoa, Spain, 2003-2008).

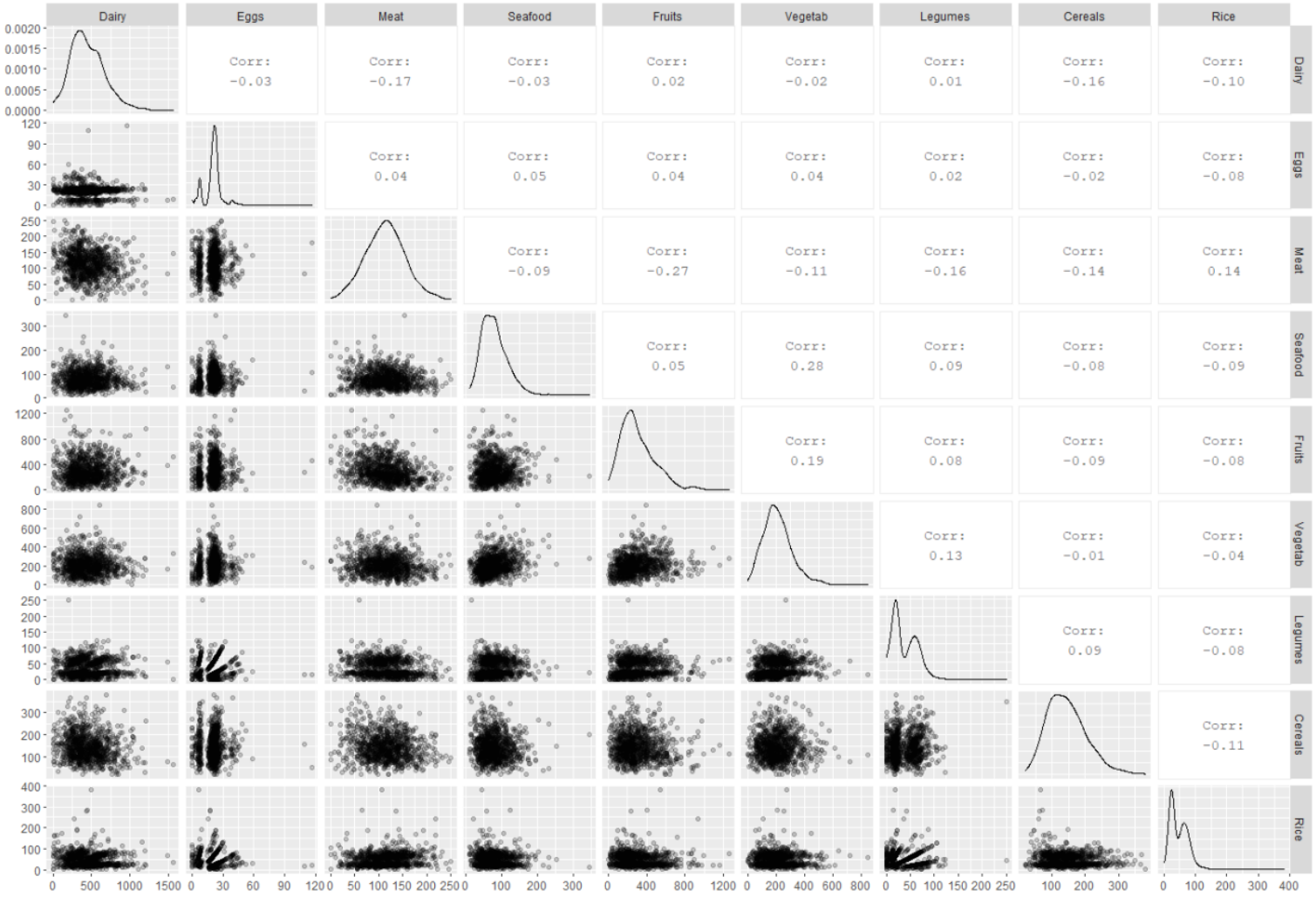
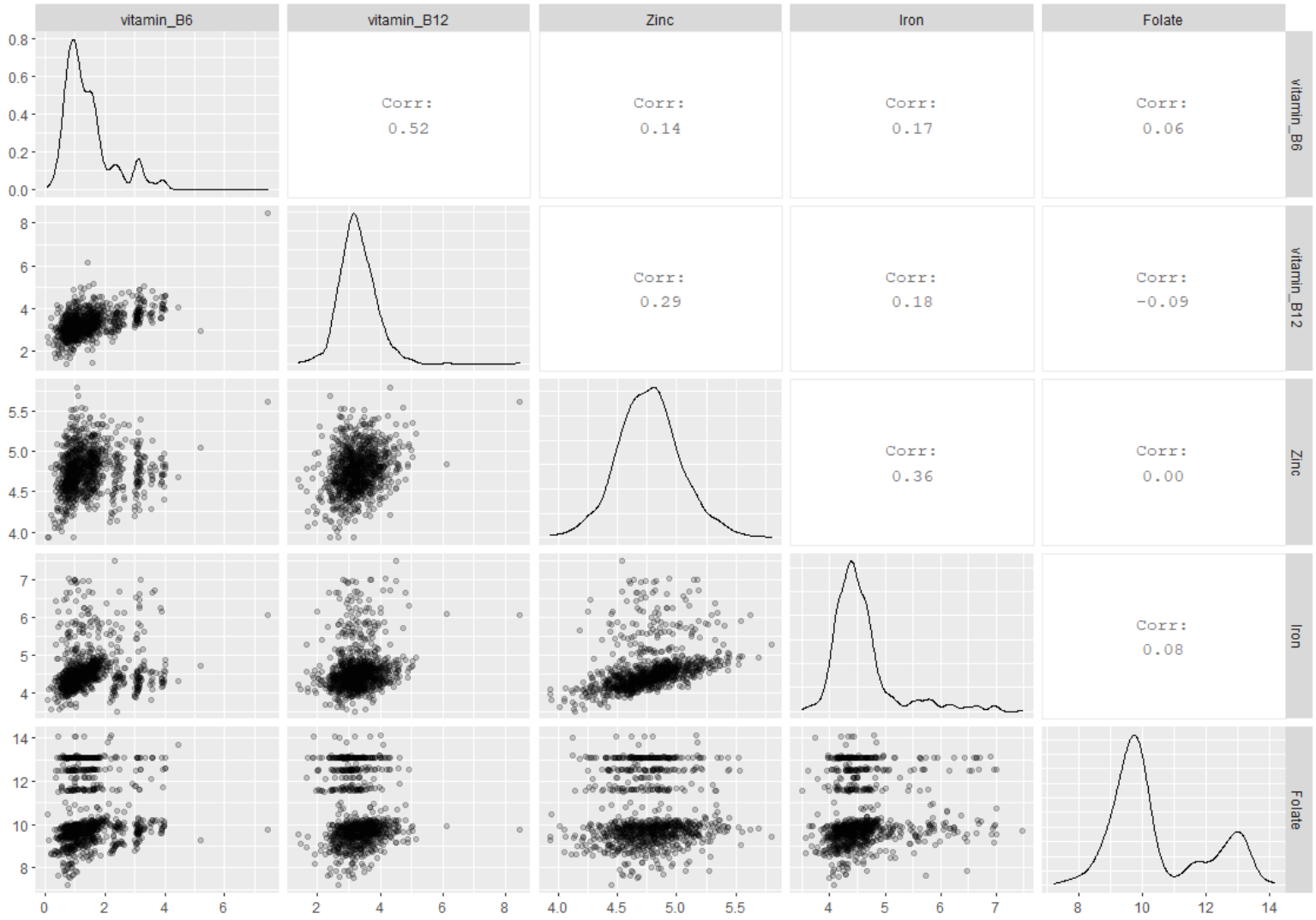
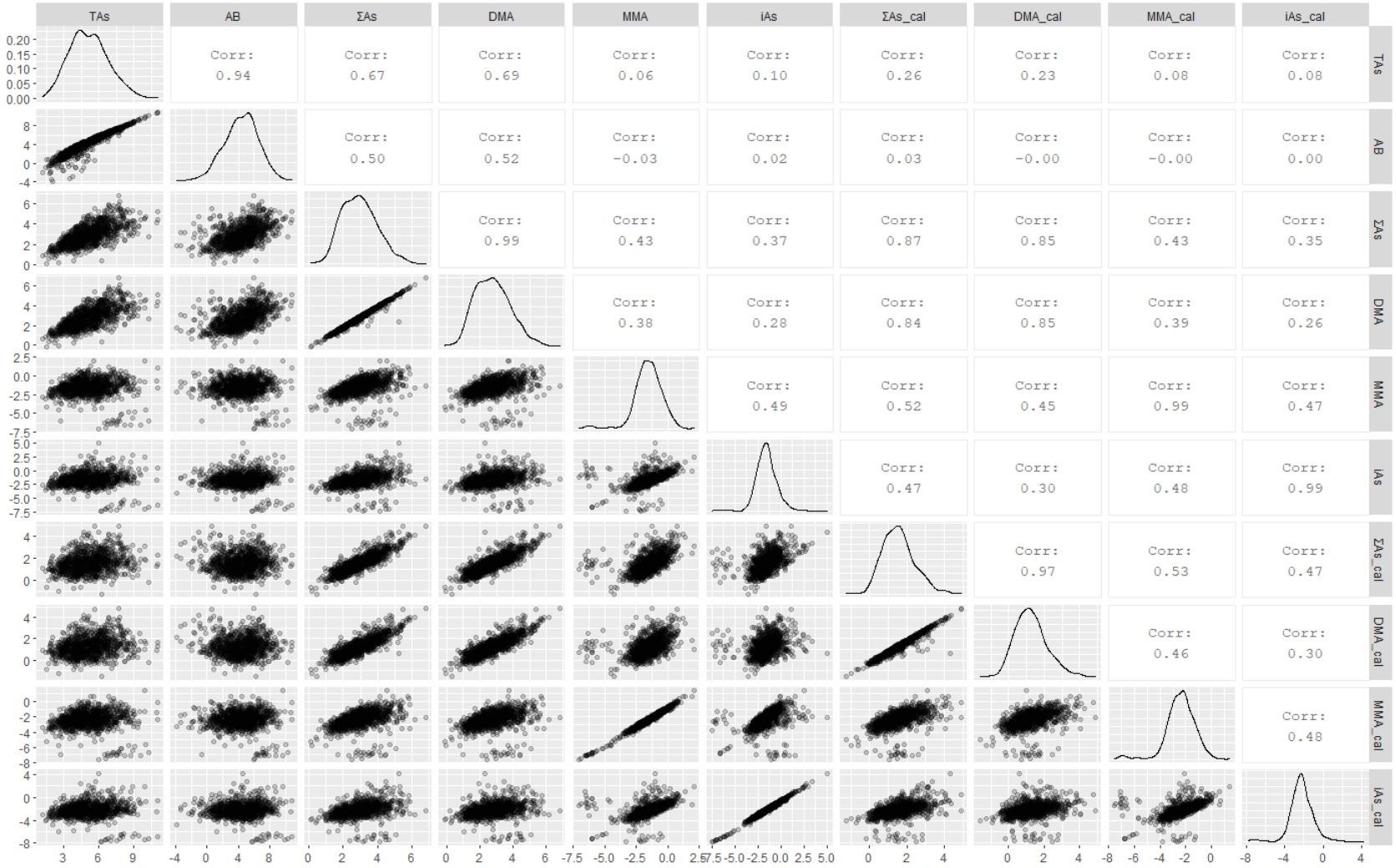


Figure S2 Correlations between energy adjusted log2-transformed estimated nutrients intake. INMA Project (Valencia and Gipuzkoa, Spain, 2003-2008).



Note: Vitamins B_6 and B_{12}, zinc and iron are expressed in mg/day. Folate is expressed in µg/day.

Figure S3 Correlation between log2-transfomed measured and calibrated[a] As concentrations (µg/g creatinine) . INMA Project (Valencia and Gipuzkoa, Spain, 2003-2008).



Note: Corr, Pearson correlation; AB, arsenobetaine; TAs, Total As; TiAs, Total inorganic iAs (sum of DMA, MMA and unmethylated iAs); DMA, dimethylated arsenic; MMA, monomethylated arsenic; iAs, unmethylated inorganic As; cal, calibrated As concentrations. All As concentrations were corrected by urinary creatinine.

[a]As metabolites concentrations corrected by arsenobetaine.

Table S2: Geometric mean (95% confident intervals) of total As, total inorganic As, arsenobetaine and As metabolites concentrations of adjusted by creatinine by characteristics of participants. INMA Project (Valencia and Gipuzkoa. Spain. 2003-2008).

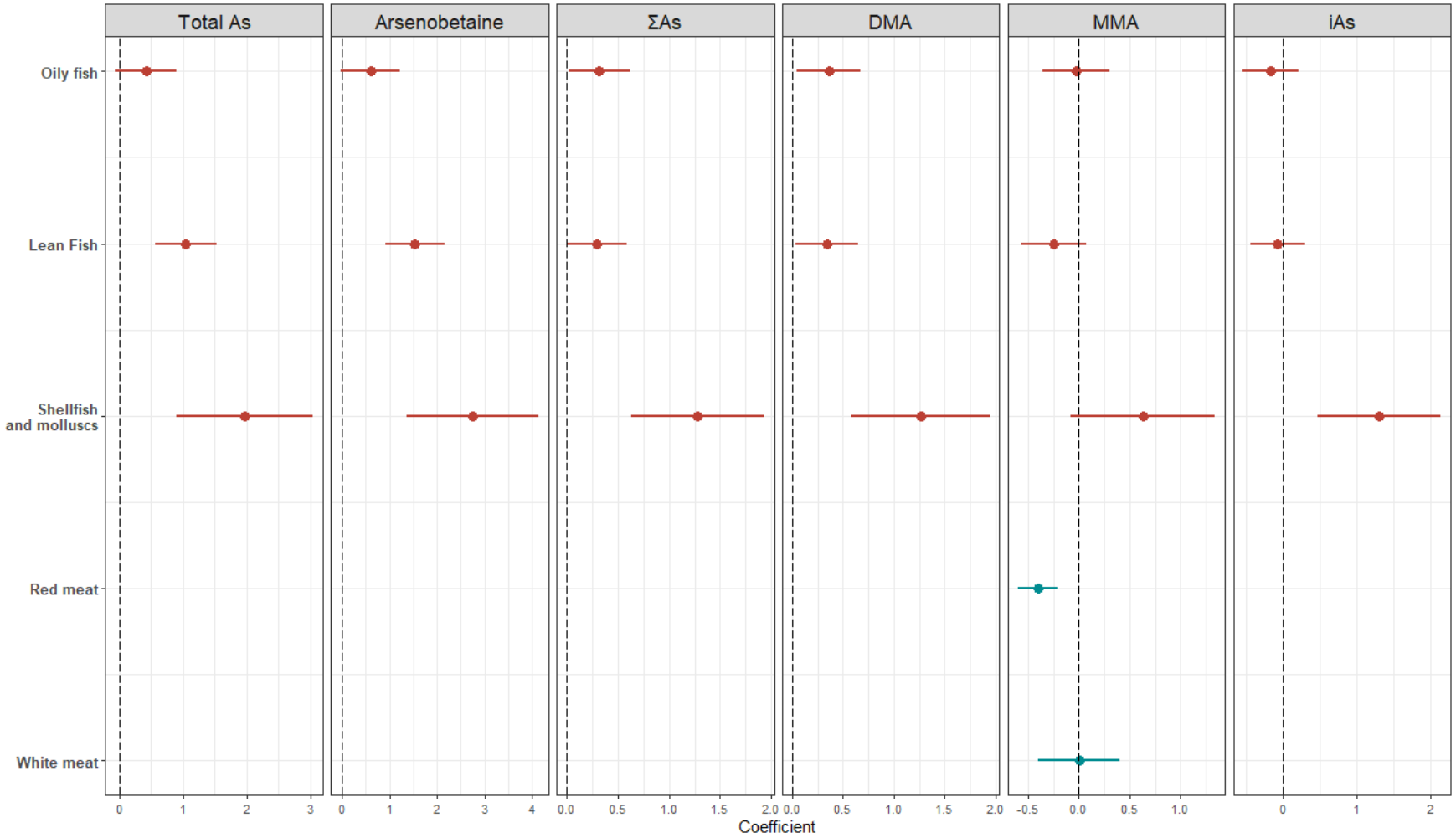
	TAs (µg/g creatinine)		p[a]	ΣAs (µg/g creatinine)		p[a]	AB (µg/g creatinine)		p[a]	DMA (µg/g creatinine)		p[a]	MMA (µg/g creatinine)		p[a]	iAs (µg/g creatinine)		p[a]
Area of study																		
Gipuzkoa	43.18	(38.49, 48.40)	<0.01	7.57	(7.09, 8.09)	0.41	26.32	(22.71, 30.49)	<0.01	6.78	(6.32, 7.27)	0.80	0.26	(0.24, 0.28)	<0.01	0.27	(0.25, 0.30)	<0.01
Valencia	31.13	(28.45, 34.07)		7.86	(7.42, 8.32)		16.81	(14.87, 19.02)		6.86	(6.46, 7.28)		0.41	(0.39, 0.43)		0.38	(0.35, 0.40)	
Age (years)																		
<25	21.35	(16.40, 27.78)	<0.01	6.74	(5.71, 7.96)	0.38	8.96	(6.04, 13.30)	<0.01	5.91	(4.97, 7.04)	0.40	0.38	(0.32, 0.45)	0.43	0.35	(0.30, 0.40)	0.66
25-29	34.55	(30.50, 39.13)		7.73	(7.15, 8.36)		19.26	(16.31, 22.74)		6.81	(6.28, 7.39)		0.34	(0.31, 0.37)		0.34	(0.32, 0.38)	
30-34	38.93	(35.08, 43.21)		7.92	(7.43, 8.46)		23.68	(20.70, 27.08)		6.98	(6.53, 7.47)		0.34	(0.32, 0.37)		0.33	(0.30, 0.36)	
≥35	36.36	(30.10, 43.91)		7.72	(6.94, 8.58)		20.09	(15.61, 25.85)		6.83	(6.12, 7.63)		0.32	(0.28, 0.36)		0.31	(0.27, 0.36)	
Place of birth																		
Spain	36.83	(34.20, 39.67)	<0.01	7.68	(7.35, 8.03)	0.07	22.17	(20.18, 24.37)	<0.01	6.78	(6.48, 7.11)	0.09	0.33	(0.32, 0.35)	0.01	0.32	(0.31, 0.34)	0.01
LatinAmerican	20.88	(15.71, 27.74)		9.52	(7.52, 12.04)		4.13	(2.44, 6.99)		8.27	(6.47, 0.57)		0.48	(0.39, 0.60)		0.47	(0.37, 0.60)	
Other	28.37	(16.70, 48.21)		6.77	(5.20, 8.82)		15.15	(7.56, 30.34)		5.79	(4.37, 7.68)		0.38	(0.31, 0.47)		0.35	(0.24, 0.51)	
BMI before pregnancy (Kg/m^2)																		
Healthy (18.5–<25)	37.59	(34.59, 40.86)	0.09	7.68	(7.35, 8.03)	0.15	21.60	(19.34, 24.13)	0.11	7.00	(6.64, 7.39)	0.19	0.35	(0.33, 0.37)	0.37	0.34	(0.32, 0.36)	0.30
Low weight (<18.5)	33.42	(19.96, 55.95)		9.52	(7.52, 12.04)		16.95	(8.31, 34.58)		5.56	(4.43, 6.99)		0.31	(0.23, 0.41)		0.29	(0.21, 0.41)	
Overweight (25–<30)	31.22	(26.45, 36.85)		6.32	(4.94, 8.10)		17.93	(14.47, 22.23)		6.47	(5.78, 7.23)		0.33	(0.30, 0.37)		0.33	(0.29, 0.37)	
Obesity (>30)	28.64	(22.01, 37.25)		11.46	(2.99, 43.88)		14.76	(10.21, 21.34)		6.61	(5.55, 7.86)		0.30	(0.25, 0.36)		0.28	(0.23, 0.35)	
Parental social class																		
I+II (high)	38.03	(33.56, 43.09)	0.41	7.98	(7.41, 8.59)	0.01	21.88	(18.49, 25.90)	0.37	7.01	(6.49, 7.57)	0.01	0.31	(0.28, 0.34)	<0.01	0.32	(0.29, 0.36)	0.68
III	33.87	(29.68, 38.65)		6.94	(6.35, 7.58)		20.44	(17.20, 24.30)		6.10	(5.56, 6.69)		0.32	(0.29, 0.35)		0.33	(0.30, 0.36)	
IV+V (low)	34.67	(30.88, 38.93)		8.10	(7.57, 8.66)		18.68	(16.02, 21.79)		7.17	(6.69, 7.70)		0.38	(0.35, 0.41)		0.34	(0.31, 0.37)	
Educational level																		
Up to primary	31.18	(26.83, 36.24)	0.07	7.47	(6.82, 8.19)	0.69	16.70	(13.66, 20.43)	0.06	6.59	(5.99, 7.25)	0.71	0.39	(0.35, 0.43)	<0.01	0.34	(0.30, 0.37)	0.20
Secondary	34.94	(31.27, 39.04)		7.85	(7.32, 8.42)		19.98	(17.24, 23.15)		6.89	(6.40, 7.41)		0.36	(0.33, 0.38)		0.35	(0.32, 0.38)	
University	39.07	(34.66, 44.04)		7.79	(7.25, 8.36)		22.73	(19.38, 26.64)		6.90	(6.41, 7.43)		0.30	(0.27, 0.33)		0.31	(0.28, 0.34)	
Working status																		
Non-working	31.66	(26.96, 37.18)	0.10	7.69	(7.03, 8.41)	0.87	16.02	(12.77, 20.10)	0.01	6.76	(6.16, 7.42)	0.84	0.40	(0.36, 0.44)	<0.01	0.34	(0.31, 0.39)	0.46
Working	36.64	(33.83, 39.68)		7.76	(7.38, 8.15)		21.44	(19.32, 23.79)		6.84	(6.49, 7.21)		0.33	(0.31, 0.35)		0.33	(0.31, 0.35)	
Proximity to agricultural area																		
No	36.58	(33.25, 40.25)	0.37	7.54	(7.13, 7.97)	0.17	19.41	(16.81, 22.41)	0.50	7.06	(6.55, 7.60)	0.22	0.37	(0.35, 0.40)	0.01	0.35	(0.32, 0.39)	0.07
Yes	33.92	(30.42, 37.82)		8.04	(7.48, 8.63)		20.76	(18.27, 23.58)		6.66	(6.28, 7.06)		0.33	(0.31, 0.35)		0.32	(0.30, 0.34)	
Proximity to industrial area																		
No	35.56	(32.58, 38.81)	0.85	7.97	(7.55, 8.41)	0.05	21.41	(18.18, 25.21)	0.41	6.45	(5.96, 6.98)	0.10	0.31	(0.28, 0.34)	<0.01	0.30	(0.28, 0.33)	0.03
Yes	35.44	(31.21, 40.26)		7.27	(6.74, 7.84)		19.65	(17.46, 22.12)		7.00	(6.61, 7.40)		0.36	(0.34, 0.38)		0.35	(0.32, 0.37)	
Tobacco consumption																		
No	36.08	(33.30, 39.08)	0.27	7.83	(7.45, 8.22)	0.23	20.28	(18.21, 22.58)	0.67	6.91	(6.57, 7.28)	0.18	0.34	(0.32, 0.36)	0.05	0.32	(0.31, 0.35)	0.17

	TAs (µg/g creatinine)		p[a]	ΣAs (µg/g creatinine)		p[a]	AB (µg/g creatinine)		p[a]	DMA (µg/g creatinine)		p[a]	MMA (µg/g creatinine)		p[a]	iAs (µg/g creatinine)		p[a]
Yes	32.46	(27.51, 38.30)		7.30	(6.63, 8.05)		19.20	(15.53, 23.75)		6.38	(5.77, 7.06)		0.38	(0.34, 0.42)		0.36	(0.32, 0.41)	
Season of sample collection																		
Winter	37.95	(32.31, 44.59)	0.13	7.52	(6.86, 8.24)	0.04	23.11	(18.82, 28.37)	0.11	6.65	(2.06, 6.04)	0.04	0.33	(0.30, 0.37)	<0.01	0.31	(0.27, 0.35)	0.45
Spring	39.45	(34.15, 45.57)		8.57	(7.81, 9.41)		22.08	(18.27, 26.69)		7.58	(2.23, 6.87)		0.39	(0.35, 0.43)		0.34	(0.30, 0.38)	
Summer	31.79	(28.19, 35.85)		7.76	(7.19, 8.37)		17.12	(14.46, 20.27)		6.80	(2.00, 6.29)		0.36	(0.33, 0.39)		0.35	(0.32, 0.39)	
Autumn	34.42	(29.52, 40.14)		7.10	(8.50, 7.75)		19.83	(16.16, 24.34)		6.27	(2.06, 5.71)		0.28	0.25, 0.32)		0.32	(0.29, 0.36)	
Rice consumption																		
<1sv/week	36.98	(33.27, 41.10)	0.31	7.16	(6.73, 7.63)	<0.01	22.35	(19.56, 25.54)	0.05	6.32	(5.92, 6.75)	<0.01	0.28	(0.26, 0.30)	<0.01	0.29	(0.26, 0.31)	<0.01
≥1 sv/week	34.34	(31.14, 37.88)		8.35	(7.86, 8.86)		18.43	(16.08, 21.11)		7.36	(6.91, 7.83)		0.42	(0.39, 0.44)		0.38	(0.35, 0.40)	
Fish consumption																		
<1sv/week	23.75	(20.59, 27.39)	<0.01	6.87	(6.30, 7.49)	<0.01	10.49	(8.41, 13.09)	<0.01	5.98	(5.47, 6.55)	<0.01	0.37	(0.34, 0.40)	0.13	0.34	(0.31, 0.38)	0.69
1 sv/week	36.74	(32.96, 40.95)		7.45	(6.99, 7.93)		22.14	(19.28, 25.42)		6.56	(6.14, 7.01)		0.32	(0.30, 0.35)		0.32	(0.30, 0.35)	
>1 sv/week	44.74	(39.62, 50.53)		8.79	(8.12, 9.52)		28.03	(24.21, 32.46)		7.82	(7.20, 8.49)		0.35	(0.32, 0.38)		0.33	(0.30, 0.36)	

Note: TAs, Total As; ∑, sum of measured DMA, MMA and iAs); DMA, dimethylarsinic acid; MMA, monomethylarsonic acid; iAs, inorganic As; BMI, Body mass index; SC, Social Class; sv, serving.

[a] p-value from ANOVA F-test

Figure S4 Fish and meat sub-categories associated with maternal urinary As, arsenobetaine and As metabolites concentrations



Note: All models were adjusted by area of study, creatinine, and country of birth. Additionally, TAs model was adjusted by age and season of sample collection, legumes and rice consumption; AB model was adjusted by age, season of sample collection, legumes and eggs consumption; ∑As model was adjusted by season of sample collection, parental social class, vegetables and rice consumption; DMA model was adjusted by season of sample collection, parental social class and vegetables and rice consumption; MMA model was adjusted by season of sample collection, parental social class, pre-pregnancy body mass index, weeks of gestation at sampling,residential proximity to agricultural area and vegetables and rice consumption; iAs model was adjusted by pre-pregnancy body mass index and legumes, rice and other cereals consumption.
Sample used of all models were n= 1005, except for log2 MMA model (n=994). All dietary variables were expressed in grams per day.

Table S3: Bivariate linear regression between methylation efficiency (measured by calibrated[1] percentage of As metabolites in maternal urine and principal component 1 and 2 of PCA) and estimated nutrients intake (adjusted by calories) and essential and toxics elements factors. INMA Project (Valencia and Gipuzkoa, Spain, 2003-2008).

	Calibrated %DMA[a,b]		Calibrated %MMA[a,b]		Calibrated %iAs[a,b]		PC1		PC2	
	Beta (95%CI)	P[c]	Beta (95%CI)	P[c]	Beta (95%CI)	P[c]	Beta (95%CI)	P[c]	Beta (95%CI)	P[c]
Estimated nutrients daily intake										
Estimated maternal zinc intake[d,e]	9.34 (0.29, 18.40)	0.04	-6.25 (-13.81, 1.30)	0.10	-9.30 (-19.25, 0.66)	0.07	0.14 (0.01, 0.28)	0.04	0.02 (-0.05, 0.09)	0.64
Estimated maternal iron intake[d,e]	-2.26 (-6.44, 1.91)	0.29	-1.08 (-4.57, 2.40)	0.54	3.11 (-1.48, 7.70)	0.18	-0.03 (-0.10, 0.03)	0.31	0.02 (-0.01, 0.05)	0.16
Estimated maternal folate intake[d,e]	-0.24 (-1.89, 1.40)	0.77	-0.79 (-2.16, 0.58)	0.26	0.54 (-1.27, 2.35)	0.56	-0.003 (-0.03, 0.02)	0.83	0.01 (-0.003, 0.02)	0.14
Estimated maternal vitamin B_6 intake[d,e]	1.43 (-1.64, 4.50)	0.36	-1.83 (-4.39, 0.73)	0.16	-1.51 (-4.88, 1.87)	0.38	0.03 (-0.02, 0.07)	0.28	0.01 (-0.01, 0.03)	0.40
Estimated maternal vitamin B_{12} intake[d,e]	0.85 (-3.35, 5.05)	0.69	-1.01 (-4.51, 2.49)	0.57	-0.97 (-5.58, 3.65)	0.68	0.02 (-0.05, 0.08)	0.63	0.005 (-0.03, 0.04)	0.77
Other essential and toxic elements										
Maternal urine cadmium (µg/L)[e]	-0.02 (-1.92, 1.88)	0.99	1.48 (-0.09, 3.05)	0.07	-0.87 (-2.96, 1.23)	0.42	0.001 (-0.03, 0.03)	0.94	-0.02 (-0.03, -0.003)	0.02
Maternal serum selenium (µg/L)	0.03 (-0.24, 0.30)	0.81	-0.13 (-0.35, 0.10)	0.27	-0.05 (-0.35, 0.25)	0.76	0.001 (-0.003, 0.005)	0.65	0.001 (-0.001, 0.003)	0.37
Maternal serum manganese (µg/L)[e]	0.78 (-5.34, 6.91)	0.80	-0.63 (-5.71, 4.45)	0.81	-0.29 (-7.06, 6.48)	0.93	0.009 (-0.1, 0.10)	0.85	0.005 (-0.04, 0.05)	0.85
Maternal serum ferritin (mg/L)	1.02 (-1.40, 3.43)	0.41	-0.71 (-2.78, 1.37)	0.50	-0.01 (-0.03, 0.02)	0.68	0.01 (-0.02, 0.05)	0.50	0.004 (-0.02, 0.02)	0.67

Note: 95%CI, 95% confidence intervals; %DMA, percentage of dimethylarsinic acid; % MMA, percentage of monomethylarsonic acid; %iAs, percentage of inorganic As. PCA, principal component analysis; PC1, principal component 1; PC2, principal component 2.

The percentages of each metabolites were calculated: levels of calibrated metabolite/ (calibrated DMA + calibrated MMA+ calibrated unmethylated iAs

[a] Calibrated percentages were calculated with As metabolites concentrations corrected by arsenobetaine and creatinine concentrations; [b]Probit- transformed; [c] p-value from ANOVA F- test; [d]Estimated nutrients daily intake from the diet and the supplementation.; [e]log2-transformed.

The models are adjusted by area of study.

Table S4 Dietary intake variables (energy adjusted) during the first trimester of pregnancy by study area. INMA Project (Valencia and Gipuzkoa, Spain, 2003-2008).

Food groups (grams per day)	Total		Valencia		Gipuzkoa		p[a]
	Mean ± SD	Median	Mean ± SD	Median	Mean ± SD	Median	
Dairy products	448.7 ± 220.7	417.9	429.2 ± 218.7	388.3	477.1 ± 220.9	451.3	<0.001
Eggs	20.4 ± 9.0	21.3	19.5 ± 9.4	20.4	21.7 ± 8.0	22.5	<0.001
Meat	113.6 ± 41.7	114.4	122.5 ± 41.1	121.0	100.6 ± 39.1	100.0	<0.001
Seafood	67.6 ± 34.9	63.1	61.8 ± 33.7	56.8	76.1 ± 34.9	72.8	<0.001
Vegetables	211.9 ± 104.6	201.5	201.9 ± 107.2	192.9	226.4 ± 99.0	213.6	<0.001
Fruits	304.7 ± 181.3	267.8	273.9 ± 174.5	236.7	349.4 ± 182.0	329.3	<0.001
Legumes	37.7 ± 26.3	27.2	28.7 ± 22.2	20.5	50.6 ± 26.5	55.5	<0.001
Rice	49.5± 33.0	42.5	60.9 ± 35.2	60.5	32.9 ± 20.4	27.1	<0.001
Other cereals	144.5 ± 59.8	137.2	135.3 ± 54.3	130.6	157.9 ± 64.7	151.3	<0.001

Note: SD, standard deviation.

Supplementary Material

Prenatal arsenic exposure, arsenic methylation efficiency, and neuropsychological development among preschool children in a Spanish birth cohort

Raquel Soler-Blasco, Mario Murcia, Manuel Lozano, Blanca Sarzo, Ana Esplugues, Gabriel Riutort-Mayol, Jesús Vioque, Nerea Lertxundi, Loreto Santa Marina, Aitana Lertxundi, Amaia Irizar, Simone Braeuer, Ferran Ballester, Sabrina Llop.

Appendix S1: Methodology

A.1.1 Description of urinary arsenic speciation analysis

The total As concentrations were determined with an inductively coupled plasma tandem mass spectrometer (ICPMS/MS, 8800, Agilent Technologies, Waldbronn, Germany) with oxygen as the reaction gas at m/z 75 → 91. An external calibration was used for quantification, from 0.05 to 100 µg As/L. The certified reference materials (CRM) SRM 1640a (Trace elements in natural water, NIST, Gaithersburg, USA, n=12) and SRM 2669 I and II (Arsenic Species in Frozen Human Urine, n=14) were used for quality control. A calibration standard was re-measured after every 10th sample to monitor the stability of the measurement. Chromatographic separation of the arsenic compounds was carried out in accordance with a previously validated method (Scheer et al. 2012). An external calibration was used for quantification. It contained arsenate, MMA, DMA and AB in the concentration range 0.05–100 µg As/L. Hydrogen peroxide (10% v/v) was added to oxidize the species. For quality control, the CRMs SRM 1640a (n= 5), SRM 2669 I (n = 8) and SRM 2669 II (n = 22) were prepared similar to the urine samples and also investigated. The 1.0 µg As/L calibration standard was injected regularly to control the stability of the measurement. Every 10th sample was also re-measured for the same purpose. Of all the samples analysed (1017), 102 were re-analysed, which represents 10%. These second measurements matched the first in 100 ± 3% of the cases. HPLC (1200, Agilent Technologies) coupled to ICPMS/MS (8800, Agilent Technologies) was employed for speciation analysis. The arsenic signal was again recorded in oxygen reaction mode at m/z 75 → 91, with the addition of CO2 for signal enhancement.

A.1.2 Description of covariables and potential confounders

Covariates collected through questionnaire during pregnancy:

Maternal and paternal age at conception (years), maternal and paternal education level (up to primary, secondary, university), maternal place of birth (Spain, other), maternal body mass index (BMI, kg/m2) before pregnancy, BMI categorized on three levels (Low and healthy weight, BMI<25; Overweight, 25≥BMI<30; Obesity, BMI≥30), parity (0, ≥1), maternal and paternal working status during pregnancy (non-worker, worker), type of area of residence (rural, non-rural), maternal tobacco consumption during pregnancy (yes, no), paternal tobacco consumption during pregnancy (yes, no), maternal alcohol consumption during pregnancy (yes, no), season of sample collection (spring, summer, winter, autumn).

Parental social class: defined from the maternal or paternal occupation during pregnancy with the highest social class, according to the Spanish adaptation of the International Standard Classification of Occupations coding system approved in 1988 (ISCO88). Class I+II included managerial jobs, senior technical staff, and commercial managers; Class III included skilled non-manual workers; and class IV+V included manual and unskilled workers.

Covariates collected through questionnaire from birth to 4-5 years of age:

Duration of breastfeeding (defined as receiving breast milk, although this could be supplemented with any food or liquid, including non-human milk, in weeks), maternal and paternal working status (non-worker, worker), maternal and paternal smoking habit (smoker, non-smoker), main care provider (mother, mother and others, other combinations without mother) and attendance at nursery (yes, no).
A proxy of the maternal verbal intelligence quotient (IQ) was assessed using the Similarities Subtest of the Weschler Adult Intelligence Scale-Third Edition (WAIS-III) (Weschler 2001) administered to the mother at the same time as the MSCA.

A.1.3 Description of the arsenic species calibration methodology

We used the mathematical method proposed by Jones et al. (2016). Arsenobetaine concentrations were used as a marker of seafood consumption. Using linear regression models, calibrated iAs, DMA and MMA concentrations were estimated by regressing the measured concentrations of iAs, MMA, and DMA on AB and creatinine concentrations (all measures were log-2 transformed) in three separate models. The new calibrated iAs, MMA and DMA concentrations were calculated by adding the residual of each metabolite model to a constant (mean level of each metabolite estimated from participants with AB<1 µg/L).

References

Jones MR, Tellez-Plaza M, Vaidya D, Grau M, Francesconi KA, Goessler W, et al. 2016. Estimation of Inorganic Arsenic Exposure in Populations with Frequent Seafood Intake: Evidence from MESA and NHANES. Am J Epidemiol 184:590–602; doi:10.1093/aje/kww097.

Scheer J, Findenig S, Goessler W, Francesconi KA, Howard B, G UJ, et al. 2012. Arsenic species and selected metals in human urine: validation of HPLC/ICPMS and ICPMS procedures for a long-term population-based epidemiological study. Anal Methods 4:406–413; doi:10.1039/C2AY05638K.

Weschler D. 2001. *Weschler Adult Intelligence Scale-III (Escala de inteligencia de Wechsler para adultos-III) (WAIS-III).* TEA Ediciones:MAdrid.

Table S1: Sociodemographic and environmental characteristics of study participants, and differences between populations included and excluded in the study. INMA Project (Valencia and Gipuzkoa, Spain. 2003-2008).

	Included population (n=807)	Excluded population[a] (n=592)	
Variables at pregnancy	N[b] (%)	N (%)	P-value[c]
Area of study			
Gipuzkoa	340 (42)	272 (46)	0.16
Valencia	467 (58)	320 (54)	
Gestational age at sampling[e]	13.0 (1.2)	13.1 (1.3)	0.18
Maternal age (years)			
<25	36 (4)	67 (12)	<0.01
25-29	263 (33)	203 (34)	
30-34	361 (45)	238 (40)	
≥35	147 (18)	84 (14)	
Maternal country of birth			
Spain	764 (95)	517 (87)	<0.01
Others	43 (5)	75 (13)	
BMI before pregnancy (Kg/m^2)			
≤25 (low and healthy weight)	610 (76)	447 (76)	1.00
25- <30 (overweight)	137 (17)	101 (17)	
≥ 30 (obesity)	60 (7)	43 (7)	
Parity			
0	446 (55)	318 (54)	0.59
≥1	361 (45)	274 (46)	
Parental social class			
I+II (high)	284 (35)	156 (26)	<0.01
III	214 (27)	133 (23)	
IV+V (low)	309 (38)	303 (51)	
Maternal educational level			
Up to primary	168 (21)	180 (31)	<0.01
Secondary	325 (40)	227 (38)	
University	313 (39)	184 (31)	
Paternal educational level			
Up to primary	168 (21)	247 (42)	<0.01
Secondary	325 (40)	244 (42)	
University	313 (39)	97 (16)	
Proximity of the residence to agricultural area			
No	469 (59)	379 (66)	0.01
Yes	329 (41)	198 (34)	
Maternal tobacco consumption[d]			
No	653 (82)	448 (78)	0.05
Yes	143 (18)	128 (22)	
Maternal alcohol consumption[d]			
No	691 (86)	493 (85)	0.50
Yes	111 (14)	87 (15)	
Rice consumption at 12 wg (grams/day)[e]	48.8 (30.5)	49.4 (35.8)	0.77
Fish consumption at 12 wg (grams/day)[e]	80.0 (35.1)	76.9 (36.0)	0.11
Vitamin B_6 intake (mg/day) [e]	3.3 (2.9)	3.2 (2.6)	0.31
Vitamin B_{12} intake (mg/day) [e]	10.4 (4.8)	10.0 (4.4)	0.13
Folate and folic acid intake (µg/day) [e]	2583.2 (3111.1)	2519.8 (3169.4)	0.71

Maternal serum ferritin			
<15 µg/L	119 (15)	120 (23)	<0.01
≥15 µg/L	613 (76)	412 (77)	
Variables until 1 year old			
Child's sex			
Female	403 (50)	267 (45)	0.10
Male	403 (50)	320 (55)	
Attendance at nursery			
No	168 (21)	97 (23)	0.51
Yes	623 (79)	327 (77)	
Variables at 5 years old			
Child's age (years)[e]	5.2 (0.7)		
Maternal working status			
Non-worker	213 (27)		
Worker	586 (73)		
Paternal working status			
Non-worker	79 (9)		
Worker	720 (91)		
Maternal tobacco consumption			
No	570 (74)		
Yes	199 (26)		
Paternal tobacco consumption			
No	519 (68)		
Yes	247 (32)		
Main caregiver			
Mother	472 (59.0)		
Mother and others	262 (33)		
Other without mother	66 (8)		
Maternal verbal intelligence quotient[e]	9.8 (3.1)		

Note: N, sample size; BMI, Body mass index; wg, weeks of gestation.

[a] Mother–child pairs included in the cohort at birth but not participating in the present study for different reasons (deaths, withdrawals, lost to follow-up, unavailability of As measurements);
[b] Missing values for some variables not included in percentages: Maternal educational level (1), Paternal educational level (6), Proximity of the residence to agricultural area (9), Proximity of the residence to industrial area (9), Maternal tobacco consumption until 12 wg (11), Maternal alcohol consumption until 12 wg (5), Rice consumption at 12 wg (5), Rice consumption at 12 wg (5), fish consumption at 12 wg (5), vitamin B6 and B12, folate, zinc and iron intake (5), child's sex (1), attendance at nursery (16), Main caregiver at 4–5 years old (7), maternal working status at 4–5 years old (8), paternal working status at 4–5 years old (14), maternal tobacco consumption at 4–5 years old (38), paternal tobacco consumption at 4–5 years old (41), maternal verbal intelligence quotient (40), maternal serum ferritin (75).
[c] p-value, comparing women's characteristics between included and non-included population using Fisher's Exact Test for categorical variables and Kruskal Wallis Test for continuous variables;
[d] tobacco and alcohol consumption until the first trimester of pregnancy;
[e] mean and standard deviation.

Table S2 Description of maternal urinary TAs and its metabolite concentrations and calibrated[a] and non-calibrated metabolite percentages. INMA Project (Valencia and Gipuzkoa, Spain. 2003-2008).

	GM (95%CI)	Min	Percentile 25th	Percentile 50th	Percentile 75th	Max
As concentrations (µg/L)						
TAs	29.24 (26.90, 31.78)	1.00	12.39	27.24	65.54	1526.17
AB	16.96(15.24. 18.88)	0.04	6.14	18.24	50.41	1287.58
ΣAs	6.35 (6.02. 6.71)	0.61	3.70	6.26	10.73	84.54
DMA	5.59(5.29. 5.92)	0.43	3.20	5.44	9.53	81.08
MMA	0.28 (0.260.30).	0.02	0.17	0.29	0.49	4.34
iAs	0.27 (0.25. 0.29)	0.01	0.17	0.27	0.45	14.07
As concentrations (µg/ g creatinine)						
TAs	35.75 (33.01. 38.73)	2.28	15.88	32.64	71.76	2123.54
AB	20.72 (18.66. 23.01)	0.07	8.12	22.04	55.12	1763.97
ΣAs	7.78 (7.41. 8.17)	1.08	4.56	7.42	12.55	111.81
DMA	6.85 (6.51. 7.21)	0.92	3.89	6.55	11.34	111.06
MMA	0.34 (0.32. 0.36)	0.01	0.23	0.35	0.55	4.17
iAs	0.33 (0.31. 0.35)	0.01	0.21	0.32	0.53	32.81
Metabolite percentages (Non-calibrated)						
%DMA	89.75 (89.25. 90.26)[b]	12.99	85.23	89.75	93.41	99.80
%MMA	5.08 (4.77. 5.32)[b]	0.05	3.23	5.08	7.23	22.33
%iAs	4.71 (4.45. 4.95)[b]	0.03	2.93	4.71	7.20	85.71
Metabolite percentages (Calibrated[a])						
%DMA	84.47 (84.00. 85.04)[b]	8.47	78.26	84.47	89.15	99.53
%MMA	7.07 (6.67. 7.40)[b]	0.13	5.01	7.07	9.82	30.79
%iAs	7.55 (7.29. 7.93)[b]	0.09	5.27	7.55	11.29	90.37

Note: TAs, Total As; ΣAs, sum of DMA, MMA and iAs; DMA, dimethylarsinic acid; MMA, monomethylarsonic acid; iAs, inorganic As; %DMA, percentage of dimethylarsinic acid; %MMA, percentage of monomethylarsonic acid; %iAs, percentage of inorganic As; µg/L: micrograms per litre. µg/g creat: micrograms per gram of creatinine; GM: Geometric mean; 95%CI: 95% confidence intervals; Min: minimum; P25th:
The percentages of each metabolite were calculated: levels of the metabolite/(DMA + MMA + iAs)*100
[a]As metabolites concentrations corrected for arsenobetaine concentrations
[b]Median (95% confidence intervals)

Table S3.1 Effect modification (beta coefficients and 95%CI and p-value of interaction **<0.05 in red bold**, **<0.01 in blue bold**) of some child and maternal factors on the association between the calibrated %DMA and the child neuropsychological development assessed by the McCarthy test scores at 4–5 years of age. INMA Project (Valencia and Gipuzkoa, Spain. 2003-2008). INMA Project (Valencia and Gipuzkoa, Spain. 2003-2008).

		General	Verbal	Percept-perf.	Quantitative	Memory	Motor	Gross motor	Fine motor	Executive function	Working memory
						Calibrated %DMA					
Main model		-0.54	-1.04	-0.66	-0.10	-0.86	-1.81	-2.31	0.28	-1.83	0.54
		(-3.21, 2.13)	(-3.62, 1.59)	(-3.29, 2.00)	(-2.70, 2.51)	(-3.58, 1.86)	(-4.48, 0.86)	(-4.90, 0.29)	(-2.27, 2.83)	(-4.59, 0.92)	(-2.03, 3.12)
Child's sex	Male	-1.78	-1.27	-0.67	-1.44	-2.02	-0.27	-1.45	2.15	-4.05	-0.83
		(-5.39, 1.83)	(-4.86, 2.32)	(-4.24, 2.89)	(-5.01, 2.13)	(-5.69, 1.65)	(-3.91, 3.38)	(-4.98, 2.09)	(-1.34, 5.64)	(-7.78, -0.32)	(-4.34, 2.68)
	Female	0.91	-0.74	-0.64	1.40	0.50	-3.56	-3.28	-1.80	0.74	2.09
		(-2.99, 4.80)	(-4.49, 3.02)	(-4.50, 3.22)	(-2.36, 5.16)	(-3.47, 4.47)	(-7.42, 0.29)	(-7.03, 0.47)	(-5.48, 1.88)	(-3.28, 4.76)	(-1.63, 5.81)
Serum Mn	<1.44 µg/L	-0.27	-1.31	1.11	-0.47	-2.27	0.74	-1.21	2.27	-1.12	0.60
		(-3.95, 3.40)	(-4.96, 2.33)	(-2.50, 4.71)	(-4.06, 3.12)	(-6.03, 1.48)	(-2.92, 4.41)	(4.84, 2.41)	(-1.21, 5.75)	(-4.90, 2.67)	(-2.96, 4.16)
	≥1.44 µg/L	-0.71	-0.28	-3.25	0.52	1.12	-4.45	-3.13	-2.46	-2.57	0.51
		(-4.64, 3.21)	(-4.06, 3.51)	(-7.20, 0.71)	(-3.31, 4.35)	(-2.87, 5.10)	(-8.33, -0.57)	(-6.88, 0.62)	(-6.27, 1.35)	(-6.62, 1.47)	(-3.28, 4.30)
Serum Se	<79.8 µg/L	-1.16	-1.47	-0.72	-0.35	-2.63	-2.18	-1.92	-0.64	-1.92	-0.26
		(-5.02, 2.70)	(-5.19, 2.26)	(-4.55, 3.11)	(-4.12, 3.43)	(-6.58, 1.32)	(-6.00, 1.64)	(-5.62, 1.79)	(-4.32, 3.04)	(-5.91, 2.08)	(-4.01, 3.50)
	≥79.8 µg/L	-0.01	-0.15	-1.12	-0.08	0.75	-1.31	-2.39	0.75	-1.91	0.92
		(-3.73, 3.70)	(-3.83, 3.53)	(-4.82, 2.59)	(-3.74, 3.57)	(-3.05, 4.56)	(-5.04, 2.42)	(-6.06, 1.28)	(-2.83, 4.34)	(-5.75, 1.93)	(-2.69, 4.54)
Urinary Zn	<363.8 µg/L	-1.13	-3.03	-0.36	0.99	-0.74	0.44	-0.19	1.36	-2.27	2.10
		(-4.91, 2.65)	(-6.67, 0.60)	(-4.06, 3.34)	(-2.69, 4.67)	(-4.62, 3.13)	(-3.30, 4.19)	(-3.83, 3.44)	(-2.20, 4.92)	(-6.19, 1.65)	(-1.52, 5.73)
	≥363.8 µg/L	-0.38	0.87	-1.61	-1.38	-1.08	-4.50	-4.86	-1.08	-1.81	-1.14
		(-4.08, 3.33)	(-2.85, 4.60)	(-5.28, 2.06)	(-5.06, 2.31)	(-4.88, 2,71)	(-8.27, -0.74)	(-8.53, -1,18)	(-4.71, 2.56)	(-5.65, 2.03)	(-4.78, 2.50)
Serum ferritin	<15 mg/l	4.51	2.39	1.34	3.13	5.77	-3.72	-9.31	3.69	1.77	3.99
		(-3.41, 12.43)	(-5.44, 10.22)	(-6.45, 9.14)	(-4.60, 10.85)	(-2.22, 13.77)	(-11.65, 4.21)	(-17.04, -1.59)	(-3.94, 11.32)	(-6.39, 9.92)	(-3.65, 11.62)
	≥15 mg/l	-2.40	-2.75	-1.40	-1.06	-2.78	-1.55	-1.31	-0.76	-2.85	-0.01
		(-5.54, 0.74)	(-5.78, 0.29)	(-4.45, 1.66)	(-4.12, 2.01)	(-5.94, 0.39)	(-4.67, 1.57)	(-4.35, 1.72)	(-3.72, 2.20)	(-6.09, 0.39)	(-3.02, 3.00)
Vit. B_6 intake	<2.3 mg	-2.11	-3.04	-2.22	-0.31	-2.07	-3.66	-3.28	-1.31	-2.48	0.33
		(-6.11, 1.90)	(-6.95, 0.88)	(-6.22, 1.78)	(-4.20, 3.57)	(-6.12, 1.98)	(-7.63, 0.31)	(-7.18, 0.63)	(-5.17, 2.55)	(-6.63, 1.67)	(-3.52, 4.17)
	≥2.3 mg	0.25	0.57	0.04	-0.37	-0.34	-0.79	-1.52	1.11	-1.71	0.32
		(-3.31, 3.81)	(-2.90, 4.03)	(-3.43, 3.51)	(-3.86, 3.12)	(-3.95, 3.27)	-4.37, 2.78)	(-4.96, 1.91)	-2.28, 4.49)	(-5.40, 1.98)	(-3.13, 3.77)
Vit. B_{12} intake	<9.4 µg	0.16	-0.14	0.49	0.68	0.35	-3.76	-3.34	-0.07	-0.61	1.08
		(-3.68, 3.99)	(-3.84, 3.57)	(-3.33, 4.31)	(-3.02, 4.38)	(-3.53, 4.23)	(-7.54, 0.02)	(-7.01, 0.33)	-3.75, 3.61)	(-4.58, 3.35)	(-2.56, 4.72)
	≥9.4 µg	-1.67	-1.90	-2.24	-1.43	-2.36	-0.51	-1.46	0.17	-3.45	-0.52
		(-5.39, 2.05)	(-5.57, 1.78)	(-5.88, 1.41)	(-5.09, 2.23)	(-6.14, 1.41)	(-4.25, 3.22)	(-5.11, 2.19)	(-3.42, 3.75)	(-7.30, 0.40)	(-4.17, 3.13)
Folate intake	<600 µg	-0.36	4.46	-6.05	0.14	0.97	-5.74	-3.29	-3.02	-0.78	0.32
		(-7.25, 6.52)	(-2.40, 11.32)	(-12.80, 0.69)	(-6.63, 6.92)	(-5.99, 7.93)	(-12.68, 1.20)	(-10.08, 3.50)	(-9.67, 3.62)	(-7.90, 6.35)	(-6.39, 7.03)
	≥600 µg	-0.85	-1.88	-0.07	-0.43	-1.43	-1.44	-2.13	0.58	-2.26	0.31
		(-3.74, 2.04)	(-4.68, 0.91)	(-2.92, 2.78)	(-3.24, 2.38)	(-4.37, 1.50)	(-4.32, 1.44)	(-4.92, 0.65)	(-2.18, 3.34)	(-5.26, 0.73)	(-2.47, 3.10)

Note: Percept-perf , perceptual-performance; Mn, manganese; Se, selenium; Zn, zinc; Vit, vitamin

Table S3.2 Effect modification (beta coefficients and 95%CI and p-value of interaction **<0.05 in red bold**, **<0.01 in blue bold**) of some child and maternal factors on the association between the calibrated %MMA and the child neuropsychological development assessed by the McCarthy test scores at 4–5 years of age. INMA Project (Valencia and Gipuzkoa, Spain. 2003-2008). INMA Project (Valencia and Gipuzkoa, Spain. 2003-2008).

		General	Verbal	Percept-perf.	Quantitative	Memory	Motor	Gross motor	Fine motor	Executive function	Working memory
						Calibrated %MMA					
Main model		-1.51	-1.42	-0.57	-2.35	**-3.34**	0.43	0.21	-0.91	0.04	-2.40
		(-4.88, 1.87)	(-4.62, 1.78)	(-3.90, 2.76)	(-5.56, 0.87)	**(-6.72, 0.05)**	(-2.83, 3.70)	(-2.97, 3.39)	(-4.06, 2.24)	(-3.47, 3.55)	(-5.56, 0.76)
Child's sex	Male	-1.10	-0.12	-1.91	0.20	-1.86	-1.74	-1.63	-2.83	0.42	-0.49
		-5.60, 3.40)	(-4.49, 4.25)	(-6.34, 2.51)	(-4.25, 4.66)	(-6,37, 2.66)	(-6.24, 2.76)	(-5.99, 2.73)	(-7.15, 1.49)	(-4.26, 5.10)	(-4.87, 3.89)
	Female	-2.01	-2.83	1.10	-4.93	-5.12	2.68	2.17	1.14	-0.43	-4.35
		(-6.96, 2.95)	(-7.37, 1.72)	(-3.82, 6.02)	(-9.41, 0.44)	(-10.07, -0.17)	(-1.93, 7.29)	(-2.32, 6.67)	(-3.32, 5.61)	(-5.58, 4.73)	(-8.77, 0.08)
Serum Mn	<1.44 µg/L	-1.81	0.44	**-3.82**	-4.03	-2.14	-1.08	-0.77	-1.96	-0.85	-3.56
		(-6.72, 3.10)	(-4.14, 5.01)	**(-8.57, 0.93)**	(-8.67, 0.62)	(-7.03, 2.74)	(-5,76, 3.59)	(-5.37, 3.83)	(-6.44, 2.53)	(-5.94, 4.24)	(-8.17, 1.05)
	≥1.44 µg/L	-1.10	-3.60	**3.34**	-1.08	-4.97	1.34	0.24	0.02	0.93	-1.42
		(-5.78, 3.59)	(-8.08, 0.88)	**-1,41, 8.09)**	(-5.54, 3.38)	(-9.71, -0.22)	(-3.21, 5.89)	(-4.21, 4.68)	(-4.46, 4.50)	(-3.93, 5.78)	(-5.82, 2 .97)
Serum Se	<79.8 µg/L	0.63	-1.02	0.91	-3.08	-1.89	0.83	-1.72	2.14	1.67	-2.96
		-4.50, 5.76)	(-5.53, 3.50)	(-4.06, 5.89)	(-7.67, 1.51)	(-6.96, 3.17)	(-3.83, 5.49)	(-6.20, 2.76)	(-2.41, 6.68)	(-3.67, 7.01)	(-7.51, 1.59)
	≥79.8 µg/L	-2.74	-2.03 (-6.56,	-1.03	-1.39	-4.53	-0.18	1.48	-3.67	-0.82	-1.49
		(-7.22, 1.75)	2.50)	(-5.59, 3.53)	(-5.93, 3.15)	(-9.15, 0.10)	(-4.73, 4.37)	(-3.07, 6.03)	(-8.09, 0.74)	(-5.49, 3.85)	(-5.99, 3.01)
Urinary Zn	<363.8 µg/L	-1.76	-0.01	-0.72	-2.96	-4.13	**-3.44**	**-2.32**	**-4.06**	0.74	-2.43
		(-6.15, 2.64)	(-4.13, 4.10)	(-5.11, 3.68)	(-7.10, 1.19)	(-8.61, 0.35)	**(-7.64, 0.75)**	**(-6.38, 1.74)**	**(-8.14, 0.02)**	(-3.82, 5.31)	(-6.49, 1.62)
	≥363.8 µg/L	-1.08	-2.57	1.53	-0.61	-1.50	**7.01**	4.83	**4.22**	-0.82	-1.71
		(-6.21, 4.04)	(-7.72, 2.58)	(-3.54, 6.60)	(-5.77, 4.55)	(-6.73, 3.73)	**(1.86, 12.16)**	(-0.33, 10.00)	**(-0.76, 9.21)**	(-6.14, 4.50)	(-6.82, 3.40)
Serum ferritin	<15 mg/l	-0.15	5.93 (-3.39,	-1.80	-4.86	-4.00	5.86	7.74	-2.09	2.44	-5.22
		(-11.52, 1.23)	15.25)	(-11.91, 8.31)	(-14.87, 5.15)	(-14.38, 6.37)	(-4.45, 16.17)	(-1.50, 16.98)	(-12.06, 7.89)	(-9.31, 14.20)	(-15.10, 4.65)
	≥15 mg/l	-1.38	-1.71	-0.03	-2.34	-3.60	-1.00	-1.09	-1.42	0.26	-2.19
		(-5.12, 2.35)	(-5.28, 1.86)	(-3.70, 3.65)	(-5.92, 1.25)	(-7.34, 0.15)	(-4.62, 2.62)	(-4.65, 2.48)	(-4.89, 2.05)	(-3.60, 4.12)	(-5.71, 1.33)
Vit. B_6 intake	<2.3 mg	-1.73	-2.00	-1.24	-4.10	-4.68	0.18	0.30	-2.25	-0.26	-3.96
		(-6.38, 2.93)	(-6.39, 2.39)	(-5.78, 3.30)	(-8.45, 0.25)	(-9.29, -0.06)	(-4.22, 4.59)	(-4.08, 4.67)	(-6.48, 1.98)	(-5.11, 4.59)	(-8.26, 0.34)
	≥2.3 mg	-0.59	-0.39	0.81	0.22	-1.12	1.39	0.10	1.18	0.75	-0.24
		(-5.38, 4.19)	(-4.98, 4.19)	(-3.96, 5.59)	(-4.40, 4.83)	(-5.91, 3.67)	(-3.36, 6.13)	(-4.42, 4.62)	(-3.45, 5.82)	(-4.23, 5.73)	(-4.78, 4.30)
Vit. B_{12} intake	<9.4 µg	-3.10	-2.15	-2.02	-3.96	-5.86	1.62	0.91	-1.20	-1.58	-3.79
		(-7.60, 1.39)	(-6.51, 2.22)	(-6.56, 2.53)	(-8.30, 0.38)	(-10.42, -1.30)	(-2.77, 6.01)	(-3.43, 5.26)	(-5.50, 3.10)	(-6.26, 3.10)	(-8.07, 0.48)
	≥9.4 µg	1.18	-0.20	1.51	0.03	0.33	-0.31	-0.62	-0.27	2.43	-0.42
		-3.77, 6.13)	(-4.80, 4.40)	(-3.28, 6.30)	(-4.57, 4.63)	(-4.53, 5.18)	(-5.05, 4.42)	(-5.18, 3.94)	(4.83, 4.29)	(-2.73, 7.58)	(-4.98, 4.15)
Folate intake	<600 µg	-2.80	-8.33	4.49	-1.83	-4.41	3.39	0.38	2.19	-0.94	-1.52
		-11.21, 5.62)	(-16.54, -0.12)	(-3.99, 12.97)	(-9.89, 6.22)	(-12.71, 3.90)	(-4.90, 11.67)	(-7.72, 8.49)	(-6.13, 0.52)	(-9.71, 7.83)	(-9.49, 6.45)
	≥600 µg	-0.86	0.09	-1.19	-2.12	-2.66	0.31	0.22	-1.21	0.46	-2.36
		(-4.52, 2.81)	(-3.36, 3.54)	(-4.78, 2.41)	(-5.60, 1.35)	(-6.33, 1.00)	(-3.21, 3.83)	(-3.20, 3.65)	(-4.62, 2.19)	(-3.36, 4.27)	(-5.78, 1.07)

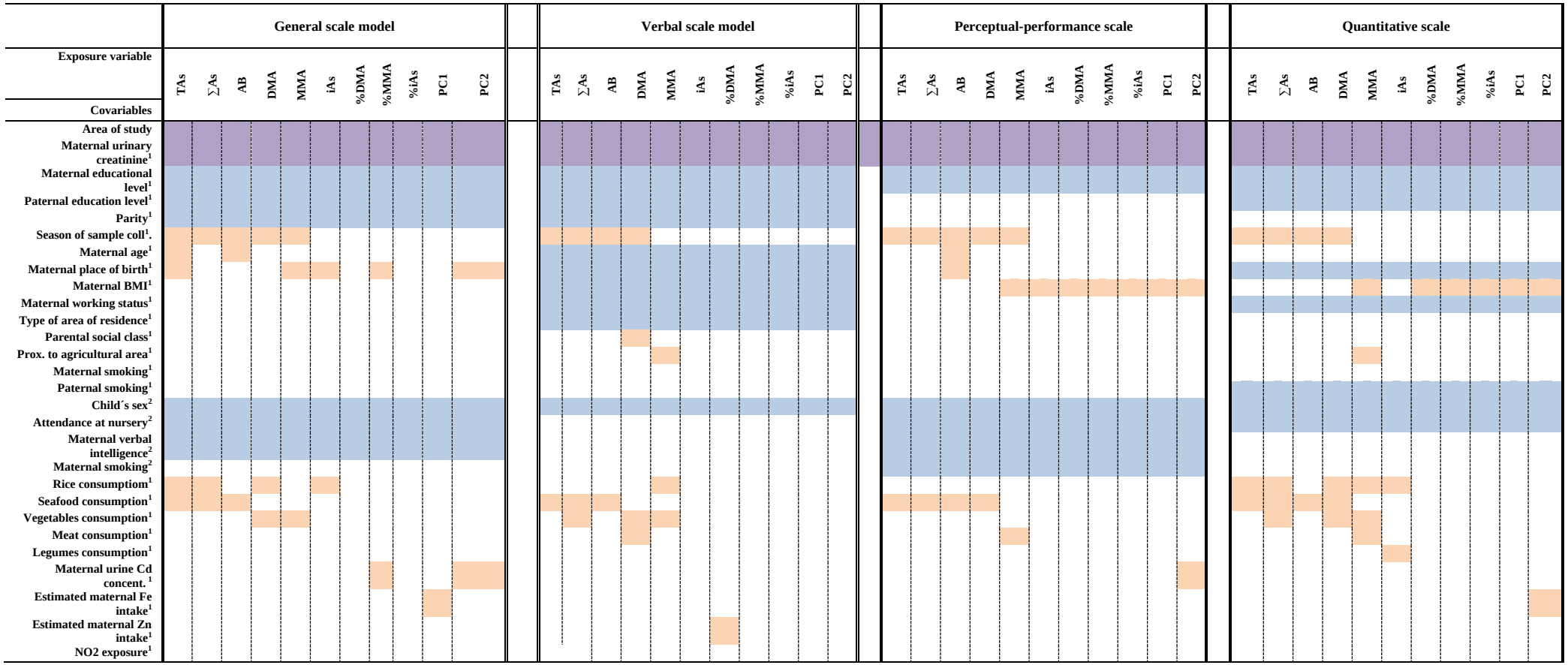
Note: Percept-perf , perceptual-performance; Mn, manganese; Se, selenium; Zn, zinc; Vit, vitamin

Table S3.3 Effect modification (beta coefficients and 95%CI and p-value of interaction **<0.05 in red bold**, **<0.01 in blue bold**) of some child and maternal factors on the association between the calibrated %iAs and the child neuropsychological development assessed by the McCarthy test scores at 4–5 years of age. INMA Project (Valencia and Gipuzkoa, Spain. 2003-2008). INMA Project (Valencia and Gipuzkoa, Spain. 2003-2008).

		General	Verbal	Percept-perf.	Quantitative	Memory	Motor	Gross motor	Fine motor	Executive function	Working memory
		Calibrated %iAs									
Main model		1.14 (-1.27, 3.56)	0.80 (-1.57, 3.16)	1.02 (-1.36, 3.40)	0.63 (-1.74, 2.99)	1.67 (-0.78, 4.13)	1.88 (-0.54, 4.30)	**2.32 (-0.03, 4.66)**	-0.10 (-2.41, 2.21)	2.02 (-0.48, 4.51)	0.14 (-2.20, 2.47)
Child's sex	Male	1.96 (-1.34, 5.26)	0.37 (-2.89, 3.64)	1.19 (-2.07, 4.45)	1.58 (-1.70, 4.85)	2.18 (-1.17, 5.53)	0.48 (-2.86, 3.82)	1.92 (-1.30, 5.14)	-2.06 (-5.26, 1.14)	3.95 (0.54, 7.36)	-0.49 (-4.87, 3.89)
	Female	0.08 (-3.46, 3.61)	1.26 (-2.16, 4.67)	0.83 (-2.64, 4.29)	-0.41 (-3.82, 3.00)	1.09 (-2.51, 4.68)	3.45 (-0.05, 6.95)	2.75 (-0.64, 6.14)	1.99 (-1.32, 5.29)	-0.21 (-3.86, 3.44)	-4.35 (-8.77, 0.08)
Serum Mn	<1.44 µg/L	1.24 (-2.10, 4.57)	0.88 (-2.43, 4.19)	-0.35 (-3.62, 2.91)	1.82 (-1.44, 5.07)	3.33 (-0.06, 6.72)	**-0.45 (-3.76, 2.87)**	1.90 (-1.39, 5.20)	**-2.47 (-5.62, 0.67)**	1.84 (-1.59, 5.27)	-3.56 (-8.17, 1.05)
	≥1.44 µg/L	0.78 (-2.76, 4.32)	0.39 (-3.02, 3.80)	2.78 (-0.75, 6.32)	-0.86 (-4.33, 2.61)	-0.55 (-4.14, 3.05)	**4.52 (1.00, 8.03)**	2.70 (-0.67, 6.06)	**3.04 (-0.37, 6.45)**	2.15 (-1.50, 5.80)	-1.42 (-5.82, 2.97)
Serum Se	<79.8 µg/L	1.47 (-2.02, 4.95)	1.36 (-2.00, 4.72)	0.74 (-2.69, 4.18)	1.09 (-2.32, 4.50)	3.05 (-0.51, 6.61)	2.37 (-1.10, 5.83)	2.45 (-0.89, 5.78)	0.13 (-3.18, 3.45)	2.05 (-1.55, 5.65)	-2.96 (-7.51, 1.59)
	≥79.8 µg/L	0.71 (-2.66, 4.07)	-0.11 (-3.44, 3.21)	1.44 (-1.89, 4.77)	0.43 (-2.89, 3.75)	0.33 (-3.11, 3.77)	1.36 (-2.01, 4.73)	2.09 (-1.23, 5.41)	-0.01 (-3.24, 3.22)	2.07 (-1.41, 5.55)	-1.49 (-5.99, 3.01)
Urinary Zn	<363.8 µg/L	1.87 (-1.50, 5.24)	1.82 (-1.40, 5.04)	1.44 (-1.85, 4.72)	-0.11 (-3.40, 3.18)	1.79 (-1.66, 5.25)	0.93 (-2.42, 4.29)	0.86 (-2.35, 4.08)	-0.18 (-3.35, 2.99)	2.29 (-1.20, 5.79)	-2.43 (-6.49, 1.62)
	≥363.8 µg/L	0.28 (-3.15, 3.71)	-0.46 (-3.93, 3.01)	0.86 (-2.53, 4.25)	1.38 (-2.04, 4.80)	1.43 (-2.07, 4.93)	3.18 (-0.31, 6.68)	4.16 (0.75, 7.57)	0.16 (-3.19, 3.51)	1.78 (-1.77, 5.34)	-1.71 (-6.82, 3.40)
Serum ferritin	<15 mg/l	**-4.27 (-10.83, 2.29)**	**-6.58 (-13.13, -0.03)**	-0.80 (-7.19, 5.59)	-2.30 (-8.73, 4.12)	**-6.34 (-12.94, 0.26)**	2.79 (-3.80, 9.38)	6.76 (0.30, 13.22)	-2.87 (-9.13, 3.40)	-1.91 (-8.67, 4.85)	-5.22 (-15.10, 4.65)
	≥15 mg/l	**3.10 (0.23, 5.97)**	**3.01 (0.26, 5.76)**	1.73 (-1.05, 4.51)	1.71 (-1.11, 4.52)	**4.10 (1.22, 6.98)**	2.01 (-0.85, 4.87)	1.72 (-1.05, 4.49)	1.11 (-1.60, 3.81)	3.12 (0.15, 6.08)	-2.19 (-5.71, 1.33)
Vit. B_6 intake	<2.3 mg	2.86 (-0.78, 6.51)	3.30 (-0.26, 6.86)	2.89 (-0.70, 6.49)	1.10 (-2.43, 4.64)	2.04 (-2.62, 6.71)	3.90 (0.30, 7.51)	3.47 (-0.07, 7.01)	2.02 (-1.45, 5.49)	2.76 (-1.01, 6.53)	-3.96 (-8.26, 0.34)
	≥2.3 mg	-0.04 (-3.27, 3.19)	-1.05 (-4.20, 2.10)	-0.12 (-3.27, 3.03)	0.58 (-2.59, 3.74)	0.50 (-2.76, 3.77)	0.55 (-2.70, 3.81)	1.43 (-1.67, 4.54)	-1.51 (-4.59, 1.57)	1.72 (-1.62, 5.07)	-0.24 (-4.78, 4.30)
Vit. B_{12} intake	<9.4 µg	0.36 (-3.26, 3.98)	-0.22 (-3.71, 3.26)	-0.03 (-3.60, 3.54)	0.22 (-3.26, 3.71)	1.30 (-2.35, 4.94)	3.94 (0.35, 7.52)	3.38 (-0.05, 6.81)	0.25 (-3.19, 3.70)	0.60 (-3.14, 4.34)	-3.79 (-8.07, 0.48)
	≥9.4 µg	1.94 (-1.32, 5.21)	1.74 (-1.51, 4.99)	2.25 (-0.95, 5.45)	1.36 (-1.87, 4.58)	2.17 (-1.14, 5.49)	0.55 (-2.73, 3.83)	1.46 (-1.75, 4.68)	-0.07 (-3.21, 3.07)	3.49 (0.11, 6.87)	-0.42 (-4.98, 4.15)
Folate intake	<600 µg	1.13 (-5.90, 8.16)	5.67 (-1.79, 13.12)	-6.70 (-14.06, 0.66)	0.08 (-6.83, 7.00)	-0.87 (-7.96, 6.22)	6.88 (-0.21, 13.97)	5.00 (-1.93, 11.94)	3.05 (-3.81, 9.90)	1.36 (-5.92, 8.64)	-1.52 (-9.49, 6.45)
	≥600 µg	1.23 (-1.34, 3.81)	1.44 (-1.06, 3.94)	0.50 (-2.02, 3.02)	0.91 (-1.60, 3.42)	2.12 (-0.49, 4.73)	1.42 (-1.15, 3.99)	1.96 (-0.52, 4.44)	-0.30 (-2.75, 2.15)	2.28 (-0.39, 4.95)	-2.36 (-5.78, 1.07)

Note: Percept-perf , perceptual-performance; Mn, manganese; Se, selenium; Zn, zinc; Vit, vitamin

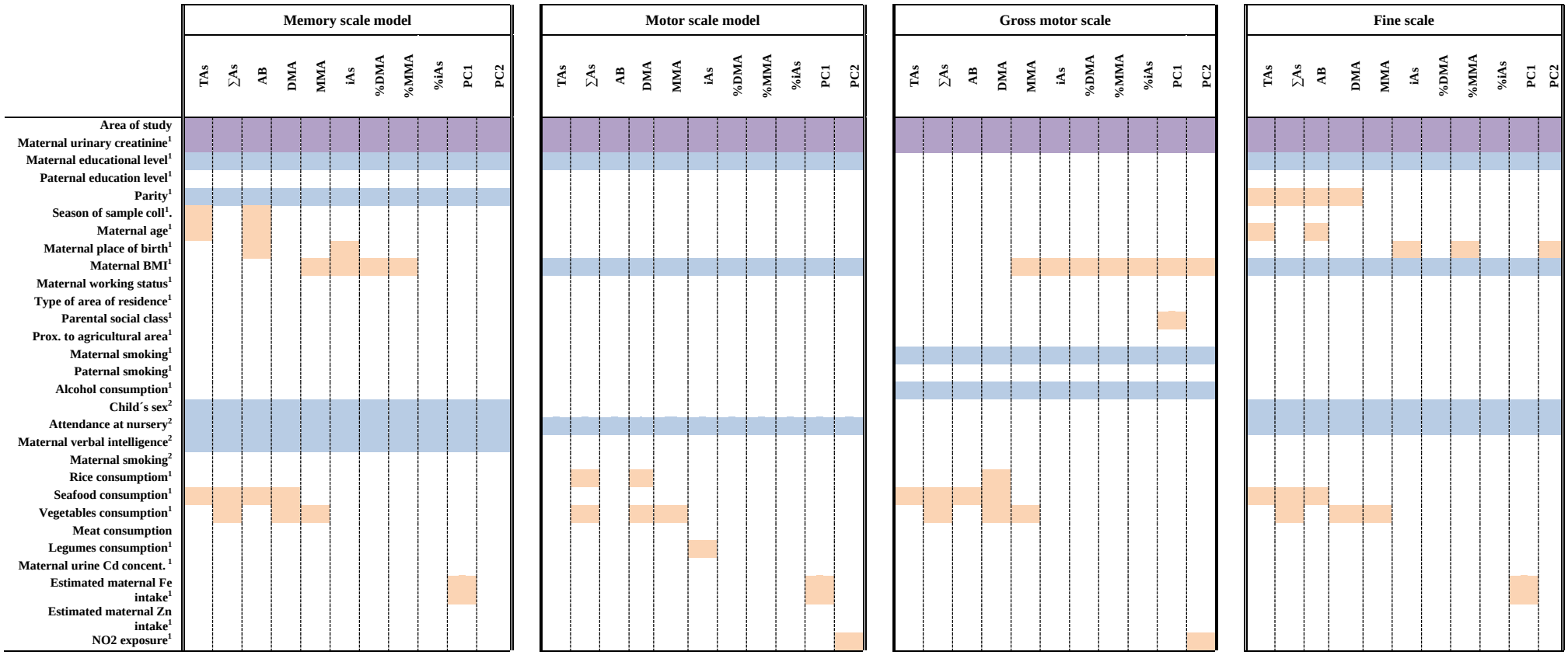
Table S4.1 Covariates and confounders included in each main model.



[1]Information collected during pregnancy [2] Information collected at 4-5 years of age

Variables included in the core model Confounders Variables included regardless of their statistical significance

Table S4.2 Covariates and confounders included in each main model.



[1]Information collected during pregnancy [2] Information collected at 4-5 years of age

Variables included in the core model Confounders Variables included regardless of their statistical significance

Table S4.3 Covariates and confounders included in each main model.

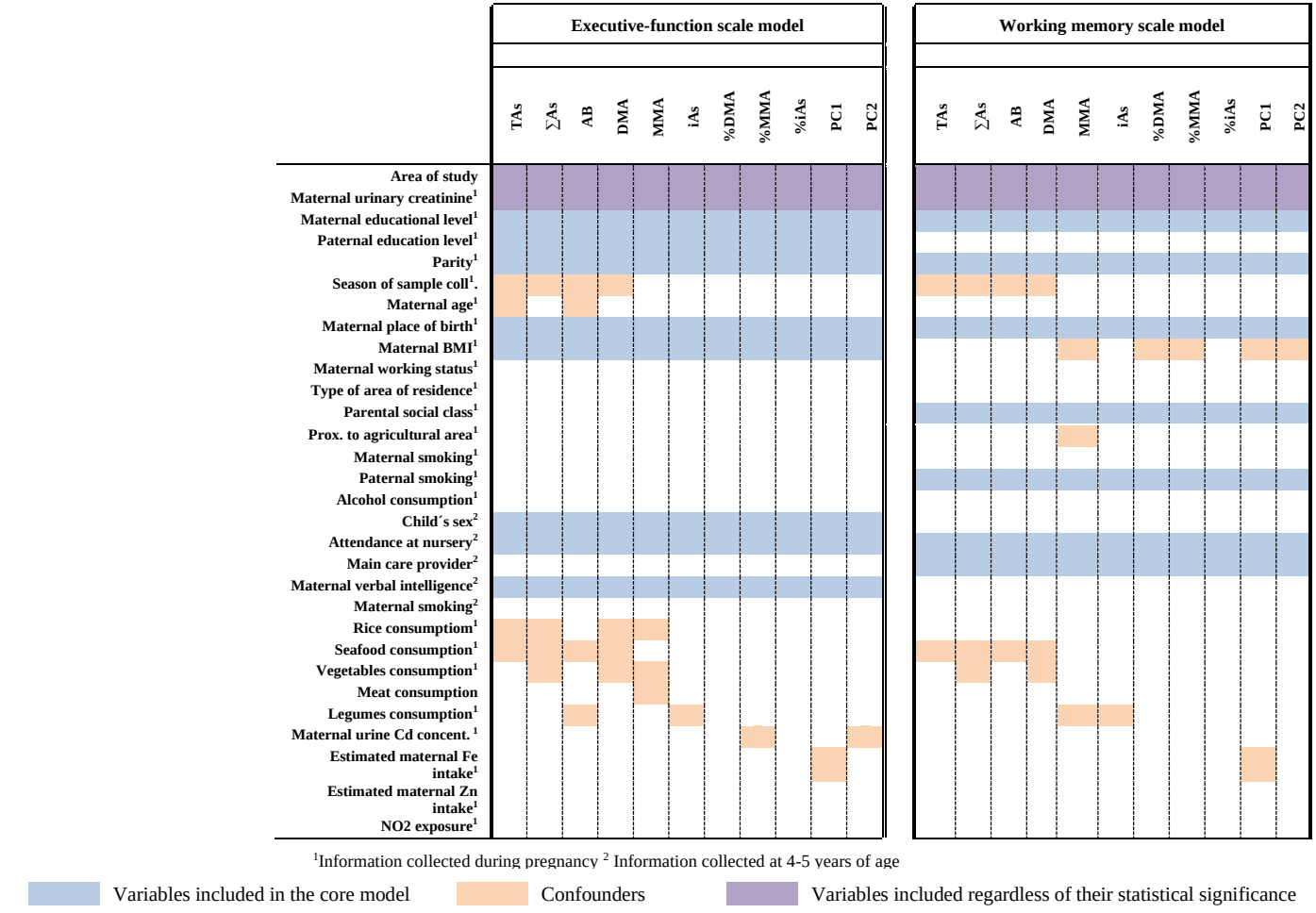


Table S5 Association (beta coefficients and 95%CI) between prenatal TAs and AB concentrations and the verbal sub-scale of the McCarthy test evaluated at 4–5 years of age: comparison effects of the main model in the total sample (model 1) and in a subsample of women in the 1st quartile of seafood consumption (model 2).

Outcome	Exposure	Model	n	Beta (95%CI)	p-value
Verbal scale	log2 TAs	1	807	0.65 (0.03, 1.27)	0.04
	log2 TAs	2	201	1.01 (-0.16. 2.19)	0.09
	log2 AB	1	807	0.59 (0.11, 1.07)	0.02
	log2 AB	2	201	0.78 (-0.06. 1.62)	0.07

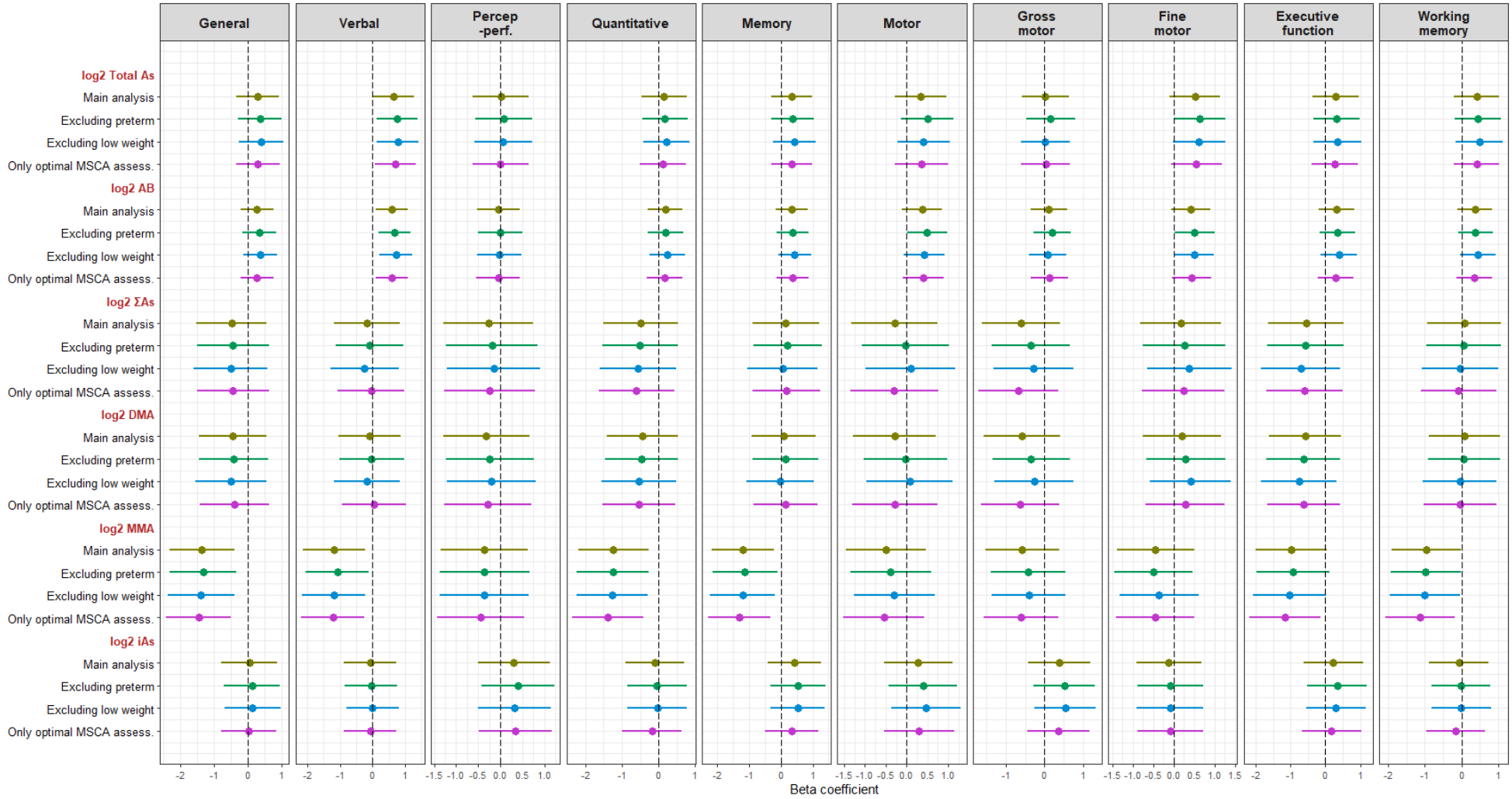
Note: TAs, Total As; AB: arsenobetaine; n= total sample used in each model.
The models were adjusted for creatinine.
Additionally, each model 1 was adjusted for different confounders and covariates (See **Tables S4.1, S4.2 and S4.3**), including adjustment by seafood consumption. Models 2 was adjusted for different confounders and covariates of model 1, without adjustment by seafood consumption.

Table S6 Association (beta coefficients and 95%CI) between prenatal MMA concentrations and several sub-scales of the McCarthy test evaluated at 4–5 years of age: comparison effects of the main model (model 1) and adjusted with maternal smoking habit at first trimester of pregnancy (model 2).

Outcome	Exposure	Model	Beta (95%CI)	p-val
General scale	log2 MMA	1	-1.37 (-2.33, -0.41)	0.01
		2	-1.32 (-2.29, -0.34)	0.01
Verbal scale	log2 MMA	1	-1.18 (-2.13, -0.23)	0.02
		2	-1.18 (-2.14, -0.22)	0.02
Quantitative scale	log2 MMA	1	-1.23 (-2.20, -0.27)	0.01
		2	-1.25 (-2.21, -0.28)	0.01
Memory scale	log2 MMA	1	-1.19 (-2.17, -0.20)	0.02
		2	-1.21 (-2.21, -0.21)	0.02
Executive function scale	log2 MMA	1	-0.98 (-2.00, 0.04)	0.06
		2	-0.98 (-2.02, 0.06)	0.07

Note: TAs, Total As; AB: arsenobetaine; n= total sample used in each model.
The models were adjusted for creatinine.
Additionally, each model 1 was adjusted for different confounders and covariates (See **Tables S4.1**, **S4.2** and **S4.3**). Models 2: model 1 + maternal smoking habit during first trimester of pregnancy.

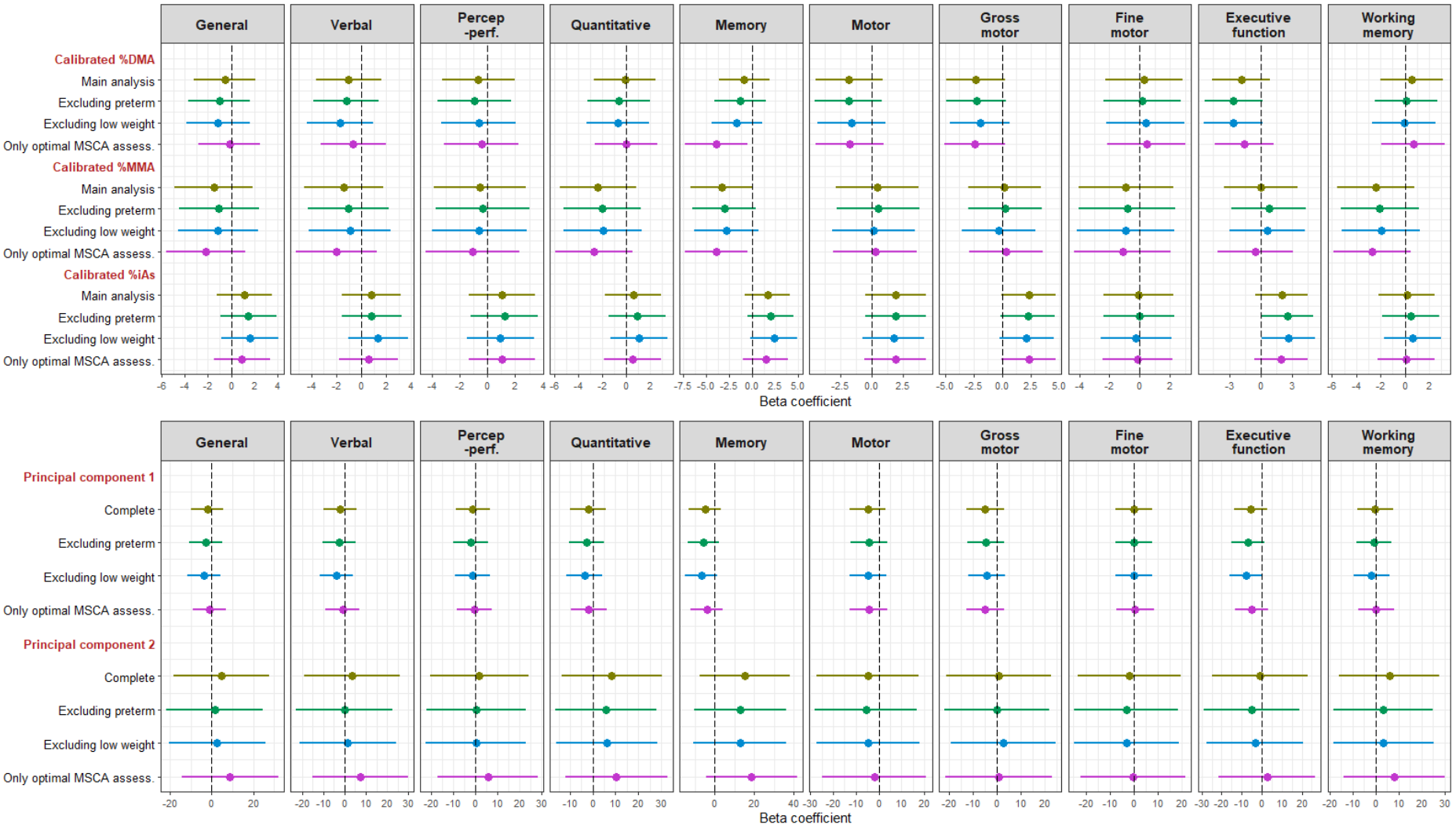
Figure S2 Sensitivity analysis: adjusted association between prenatal As concentration percentages and children's neurodevelopment assessed by the McCarthy test scores at 4–5 years of age: main models and with exclusion of special cases. INMA Project (Valencia and Gipuzkoa, Spain. 2003-2008).



Note: Percep- perf: perceptive-performance; Only optimal MSCA assess: only optimal McCarthy assessment; log2, log2 transformation. TAs, Total As; ΣAs, sum of DMA, MMA and iAs; DMA, dimethylarsinic acid; MMA, monomethylarsonic acid; iAs, inorganic As.

All models were adjusted for creatinine. Additionally, each model was adjusted for different confounders and covariates (See **Tables S4.1, S4.2 and S4.3**).

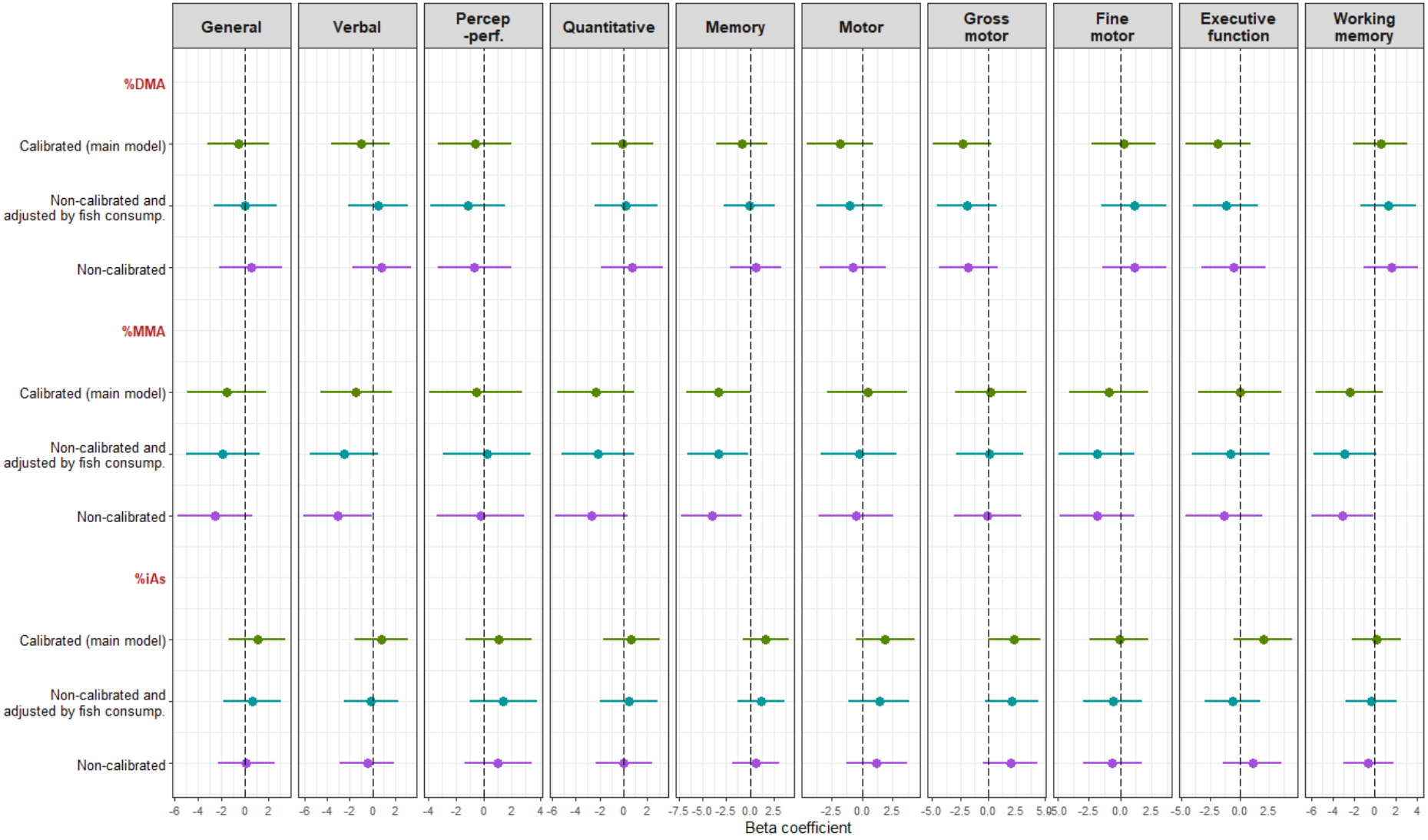
Figure S3: Sensitivity analysis: Adjusted association between prenatal As metabolism (assessed by calibrated[a] As metabolite percentages and principal components) and children's neurodevelopment assessed by the McCarthy test scores at 4–5 years of age: main models and with exclusion of special cases. (INMA Project, Spain).



Note: Percep- perf: perceptive-performance; Only optimal MSCA assess: only optimal McCarthy assessment; %DMA, percentage of dimethylarsinic acid; % MMA, percentage of monomethylarsonic acid; %iAs, percentage of inorganic As.

[a] As metabolite concentrations corrected for arsenobetaine and creatinine concentrations.
Each model was adjusted for different confounders and covariates **(See Tables S4.1, S4.2 and S4.3).**

Figure S4 Sensitivity analysis: Adjusted association between calibrated[a] and non-calibrated As metabolite percentages and children's neuropsychological development assessed by the McCarthy test scores at 4–5 years of age. INMA Project (Valencia and Gipuzkoa, Spain. 2003-2008).

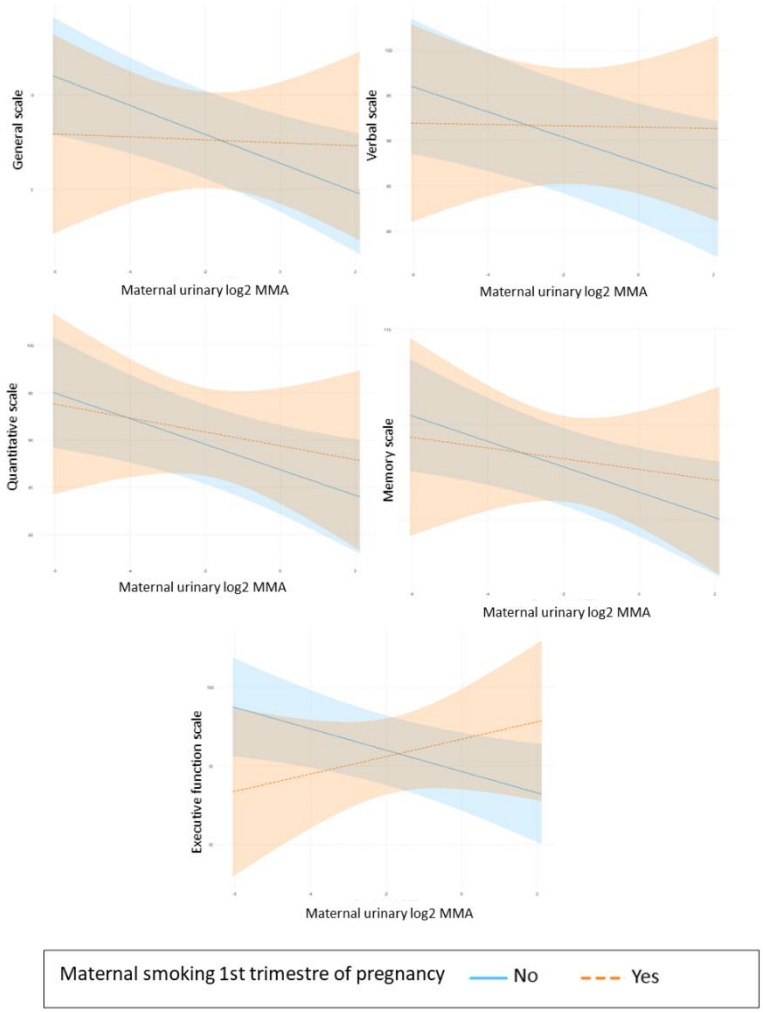


Note: Percep-perf: perceptive-performance; %DMA, percentage of dimethylarsinic acid; % MMA, percentage of monomethylarsonic acid; %iAs, percentage of inorganic As.

[a] As metabolite concentrations corrected for arsenobetaine and creatinine concentrations.

The models which use the non-calibrated percentages as the exposure variable were adjusted for creatinine. Additionally, each model was adjusted for different confounders and covariates **(See Tables S4.1, S4.2 and S4.3)**.

Figure S4 Sensitivity analysis: interactions of maternal smoking habit and log2 MMA on the association with several scales of the McCarthy test evaluated at 4–5 years of age. INMA Project (Valencia and Gipuzkoa, Spain. 2003-2008).



ANEXO 2:

Respuesta a revisores de las publicaciones

ARTÍCULO I

Title: Prenatal manganese exposure and neuropsychological development in early childhood in the INMA cohort.

Manuscript number IJHEH_2019_752

We are very grateful to the reviewers for their careful reading of our manuscript, which has allowed us to clarify some aspects of it. The specific points raised by the reviewers are addressed below. We have tracked changes in the text.

Editor

I have completed my evaluation of your manuscript. The reviewers recommend reconsideration of your manuscript following revision. I invite you to resubmit your manuscript after addressing the comments below.

We would like to thank the editor for giving us the opportunity to review our manuscript in accordance with the points raised by the reviewers.

Comments of Reviewer 1

The investigation of this paper is thorough and even though the results are null, I am glad to see the data being reported to inform future research!
When reading the paper the wording "negative but not significant" was used several times to describe the relationship between prenatal Mn and neurodevelopment. While this is true and may be appropriate for the results section, it was a little misleading in the discussion section. I wouldn't put too much stress on the negative finding since it was not statistically significant or even marginally significant. Rather, I would suggest focusing on the fact that there was a null finding (which is still very important to report).

The authors agree with the reviewer's comment and, as he/she has suggested, we have changed the wording "negative but not significant" to "a null association" in the Discussion section. In the Discussion and Conclusion sections, the authors have explained that the possible cause for this null result could be that the Mn levels in our population are in the

homeostatic ranges: "*This disparity between the results of our study and findings in other works may be due to the fact that Mn concentrations in our population (GM [95%CI] =1.50 [1.48-1.53] µg/L in maternal serum) could be within a normal/homeostatic range, in which this compound behaves as an essential element*". Furthermore, in the Discussion we have added some information about the exposure to Mn in the Spanish general population. So, the results of the present study can be contextualized in non-contaminated areas; **Discussion section, page 18**: "*Exposure to Mn through drinking water in the Spanish general population is low (approximately 99% of water supply controls were below the WHO guide value of 400 µg/L)* (Palau Miguel et al., 2008)*. In addition, the daily intake of Mn estimated in some studies is situated within the range of the adequate daily requirement proposed by the WHO (2–5 mg per day)* (Goñi and Hern, 2019; Rubio et al., 2009; World Health Organization, 1996)*".*

Since these were pregnant women, was prenatal vitamin usage considered in the models since they contain a variety of beneficial nutrients for the fetus? Maybe those who consumed foods rich in Mn also adhered to a prenatal vitamin regime which provided some protection against elevated Mn absorption.

Following the reviewer's recommendation, we have calculated a bivariate linear regression model with the variable "maternal serum Mn levels" as the outcome and the variable "multivitamin supplementation until 12th wg" (categorized as "No" and "Yes") as the predictor. Women who took multivitamins had lower Mn concentrations than women who did not take them. We have added these results to Table 1. We tested the variable for the multivariate model because the p-value in the bivariate analysis was <0.20. The variable was tested in two multivariable models (basal model and the multivariate model, which include dietary variables).

The result was the following:

Basal model

	Beta	95%CI		p-val
Maternal working status				
Non-working				
Working	-0.05	-0.11	0.01	0.08
Multivitamin supplementation until 12 wg [ref: No]	-0.05	-0.11	0.01	0.13

Model with dietary variables

	Beta	95%CI		p-val
Maternal working status				
Non-working				
Working	-0.06	-0.11	0.00	0.05
Dairy products	0.00	-0.01	0.01	0.70
Eggs[2]	-0.08	-0.34	0.18	0.53
Meat	0.01	-0.04	0.05	0.68
Seafood and shellfish	0.00	-0.07	0.06	0.92
Fruits	0.00	-0.01	0.01	0.91
Vegetables	0.00	-0.03	0.02	0.71
Nuts	0.22	0.02	0.42	0.04
Legumes	-0.05	-0.15	0.04	0.27
Potatoes	-0.03	-0.10	0.03	0.35
Cereals and bread	-0.01	-0.05	0.02	0.49
Coffee and others infusions[2]	0.01	0.00	0.02	0.08
Multivitamin supplementation until 12 wg [ref: No]	-0.05	-0.11	0.01	0.11

The variable "*multivitamin intake during the first trimester of pregnancy*" was not included in the final adjusted model because the p-value obtained in the likelihood ratio test was >0.1. Moreover, we have tested whether this variable could be an effect modifier by including the interaction between Mn and "*multivitamin intake during the first trimester of pregnancy*" in both main models (mental and psychomotor). In both cases, the interaction p-value was >0.05 (0.95 on the mental scale and 0.84 on the psychomotor scale). We have added the information about the new analysis in the Methods and Results sections.

How was missing data handled?

The percentage of missing data for the majority of the variables was below 2.5%, so we did not impute these values (the data can be seen in the following table).

Variable	N of missing data	% of the total sample
Paternal education level	8	0.68%
Maternal working status at 12 wg	2	0.17%
Child sex	2	0.17%
Nuts consumption at first trimester of pregnancy	13	1.10%
Multivitamin supplementation until 12 wg	29	2.46%

Nevertheless, the variable "maternal ferritin serum at 12 wg" had 100 missing cases (which accounts for 8.48% of the total sample). This missing data can lead to a loss of statistical power in the model where we used this variable (model 2). We have added this limitation in the Discussion section (**Discussion section, page 21**): "*Another limitation is that the maternal serum ferritin variable had 8.48% of missing data, which can lead to a loss of statistical power in the model where this variable was included.*"

The inclusion of Fe and Se is important for this study of Mn exposure. How did the Se levels relate to the general population, were they higher?

In our population, the mean of maternal serum Se levels measured in the first trimester of pregnancy was 79.8 µg/L (standard deviation: 9.5µg/L). The safe range of Se for human health has been established around 60–140 µg/L (Fairweather-Tait et al., 2011), so the levels in our population are within this safe range. Other studies have reported the Se concentrations in the Spanish general population:

Study	Area of Spain	Biomarker	Se levels, µg/L [Mean (sd)]
Sánchez (2010)	Southern Spain	Plasma	Total population: 82.7 (48.3) Women: 80.5 (44.1)
Torra (1997)	Northeast Spain	Serum	Total population: 80.7 (10.0) Women: 77.1 (12.5)
Adame (2012)	Southern Spain	Serum	Total population: 76.6 (17,3)

			Women: 67.3 (10,7)
Díaz (2001)	Canary Islands	Serum	Total population: 74.7 (25.2) Women: 75.2 (25.1)

The maternal Mn Se levels in our study were similar to those studies carried out in different areas of Spain.

Can any potential recommendations about diet during pregnancy be made based off of this work?

The authors consider that diet recommendations cannot be made based only on the results of the present study. In our context, where the exposure to Mn from water consumption and diet is low, Mn levels seem to remain within a homeostatic range. Nevertheless, we consider that more prospective epidemiological studies would be necessary in order to improve our knowledge about the safe range of Mn during pregnancy and which factors can affect this homeostatic mechanism. All this information could be useful to establish contextual dietetic recommendations.

References

Adame, E.M., Florea, D., Pérez, L.S., López, J.M., Cruz, A.P. De, 2012. Deficient selenium status of a healthy adult Spanish population. Nutr. Hosp. 27, 524–528. https://doi.org/10.3305/nh.2012.27.2.5529

Díaz, C., Henrıquez, P., Rodrıguez, E., 2001. Serum selenium concentration in a representative sample of the Canarian population. Sci. Total Environ. 65–73.

Fairweather-Tait, S.J., Bao, Y., Broadley, M.R., Collings, R., Ford, D., Hesketh, J.E., Hurst, R., 2011. Selenium in Human Health and Disease. Antioxid. Redox Signal. 14, 1337–1383. https://doi.org/10.1089/ars.2010.3275

Palau Miguel, M., Ligia Guevara Alemany, E., Moreno Seisdedos, M., 2008. Calidad de agua de consumo humano en España. Informe técnico. Trienio 2005-2006-2007. Madrid.

Rubio, C., Gutiérrez, Á.J., Revert, C., Reguera, J.I., Burgos, A., Hardisson, A., 2009. Daily dietary intake of iron , copper , zinc and manganese in a Spanish population. Int. J. Food Sci. Nutr. 60, 590–600. https://doi.org/10.3109/09637480802039822

Torra, M., Rodamilans, M., Corbella, J., 2002. Biological monitoring of environmental exposure to manganese in blood samples from residents of the city of Barcelona , Spain. Sci. Total Environ. 289, 237–241.

World Health Organization, 1996. Trace elements in human nutrition and health World Health Organization. World Health Organization, Geneva.

Comments of Reviewer 2

The study investigated prenatal exposure to Mn in the first trimester and BSID scores at age 1 year in a relatively large sample in Spain. The results are likely to be influential to the literature of Mn exposure and child neurobehavioral development. Still, several limitations need to be addressed before it is acceptable for publication.

1. The differences of exposure assessment in prior epidemiologic studies are not trivial. The manuscript, as written, mentioned blood vs serum biomarker, first trimester vs delivery or cord Mn levels, and one-time vs repeated measures. These discussions need to be strengthened to include the following:

a) The distribution of Mn in whole blood, and the reliability of blood vs serum Mn;

b) The metabolism of Mn in the body as related to Mn tissue level vs biomarker level;

c) The physiology of Mn during pregnancy and why timing of Mn exposure may matter;

d) The placenta transfer of Mn and the difference of using maternal vs cord Mn.

As the reviewer has suggested, we have added information about Mn biomarkers and metabolism during pregnancy in the Discussion:

a) and b) **Discussion section, pages 17-18:** "*After absorption, Mn is rapidly distributed to the organs through the blood. The liver is the main organ that regulates Mn levels in the body. Excess Mn is sequestered by the liver and excreted into the bile, maintaining an adequate physiological level of Mn in the plasma. For this reason, Mn has a short half-life in blood and plasma/serum, resulting in a weak relationship between indicators of Mn exposure and Mn status* (EFSA Panel on Dietetic Products Nutrition and Allergies, 2013; Zheng et al., 2011)**.** *However, Mn serum concentrations seem to be slightly sensitive to large variations in Mn intake, but this fact is not conclusive* (Institute of Medicine (US) Panel on Institute of Medicine (US) Panel on Micronutrients, 2001). *In 2013, the European Food Safety Authority concluded that, due to the efficient homeostatic mechanism, there was no reliable biomarker of Mn status, thus resulting in a weak relationship between indicators of Mn exposure and Mn status (EFSA Panel on Dietetic Products Nutrition and Allergies, 2013; Lucchini et al., 2015)*".

c) The physiology of Mn during pregnancy and why timing of Mn exposure may matter.
Discussion section, pages 17-18: we have added a paragraph explaining in greater depth the physiology of Mn during pregnancy: "*Differences between the present study and previous ones as regards sample collection could also be influencing the heterogeneity of the results. In most of the previous studies, Mn concentrations were measured in the final period of pregnancy or at birth. It has been observed that there is a tendency toward increased maternal Mn levels during pregnancy* (Spencer, 1999; Takser et al., 2004)*, probably related to a higher intestinal absorption of this element due to the physiological iron deficiency during this period* (Abbassi-Ghanavati et al., 2009; Finley, 1999)*".*

d) The placenta transfer of Mn and the difference of using maternal vs cord Mn.

Mn is an essential nutrient that is required for fetal development, so there is a good transfer of this element from mother to fetus via the placenta. In fact, the concentrations in cord blood have been observed to be consistently higher than in maternal blood (Arbuckle et al., 2016; Krachler et al., 1999; Takser et al., 2004), probably due to an active transport through the placenta (Krachler et al., 1999) or a more deficient homeostatic mechanism in fetuses than in adults (Aschner and Aschner, 2005). This fact and the medium-weak correlation between maternal Mn blood and cord blood Mn concentrations observed in several studies (Guan et al., 2013; Krachler et al., 1999; Zota et al., 2008) could be suggesting that cord blood Mn concentrations would be a better biomarker of prenatal exposure to Mn than maternal blood, but only for the last period of pregnancy due to the short half-life in blood (Agency for Toxic Substances and Disease Registry, 2012; Zheng et al., 2011). As the vulnerability of the central nervous system extends from the beginning of pregnancy until adolescence, it would be more appropriate to measure Mn in several time points during pregnancy and birth.

Information about this topic has been added to the **Discussion section (page 18 and 21 [in limitations of study])**.

2. Background exposure to Mn in Spain needs to be discussed. Comparison with literature on Mn levels in environmental media, diet, and other sources can be provided to determine the representativeness of this study sample regarding Mn exposure.

As the reviewer has suggested, we have added some information about the background exposure to Mn in Spain **(Discussion section, page 18)**: "*Exposure to Mn through drinking water in the Spanish general population is low (approximately 99% of water supply controls were below the WHO guide value of 400 µg/L)* (Palau Miguel et al., 2008)*. In addition, the daily intake of Mn estimated in some studies is situated within the range of the adequate daily requirement proposed by the WHO (2–5 mg per day)* (Goñi and Hern, 2019; Rubio et al., 2009; World Health Organization, 1996)*".*

3. Discussion is needed regarding postnatal exposure to Mn, which cannot be neglected due to its potential role influencing Mn toxicity up to first year of age.

Following the reviewer's recommendation, we have added more information about postnatal exposure to Mn in the **Discussion section, page 21:** *"As the vulnerability of the central nervous system extends from the beginning of pregnancy to adolescence, it would have been more appropriate to measure Mn at several time points during pregnancy, birth and the postnatal period. Infants are exposed to Mn mainly from their diet. As the Mn concentrations in breast milk are low* (EFSA Panel on Dietetic Products Nutrition and Allergies, 2013)*, formula milk seems to be the main contributor to infants' Mn body burden* (Frisbie et al., 2019) *Thus, both prenatal and postnatal exposure to Mn could be affecting children's neuropsychological development. In the present study we have not assessed the postnatal exposure to Mn."*

4. The dynamic of neurobehavioral development goes on beyond infancy. Interpretation of the results needs to be framed in the context of exposure levels, lack of postnatal exposure assessment, and continuum of brain development.

The authors appreciate all the reviewer´s comments and we have tried to carry out a deeper interpretation of our results, taking into account the context of the study, the metabolism of Mn in the body, and the limitations of the biomarker used. All the information about these topics has been remarked in comments 1 to 3.

References

Abbassi-Ghanavati, M., Greer, L.G., Cunningham, G., 2009. A Reference Table for Clinicians. Obstet. Gynecol. 114, 1326–1331.

Agency for Toxic Substances and Disease Registry, 2012. Toxicological profile for Manganese. Agency for Toxic Substances and Disease Registry, Georgia.

Arbuckle, T.E., Lei, C., Morisset, A., Fisher, M., Weiler, H., Mihai, C., Legrand, M., Davis, K., Ettinger, A.S., Fraser, W.D., Group, S., 2016. Maternal and fetal exposure to cadmium , lead , manganese and mercury : The MIREC study. Chemosphere 163, 270–282. https://doi.org/10.1016/j.chemosphere.2016.08.023

Aschner, J.L., Aschner, M., 2005. Nutritional aspects of manganese homeostasis. Mol. Aspects Med. 26, 353–362. https://doi.org/10.1016/J.MAM.2005.07.003

EFSA Panel on Dietetic Products Nutrition and Allergies, 2013. Scientific Opinion on Dietary Reference Values for manganese. EFSA J. 11, 1–44. https://doi.org/10.2903/j.efsa.2013.3419

Finley, J.W., 1999. Manganese absorption and retention by young women is associated with serum ferritin concentration. Am. J. Clin. Nutr. 70, 37–43.

Frisbie, S.H., Mitchell, E.J., Roudeau, S., Domart, F., Carmona, A., Ortega, R., 2019. Manganese levels in infant formula and young child nutritional beverages in the United States and France : Comparison to breast milk and regulations. PLoS One 1–23. https://doi.org/10.1371/journal.pone.0223636

Goñi, I., Hern, A., 2019. Intake of Nutrient and Non-Nutrient Dietary Antioxidants. Contribution of Macromolecular Antioxidant Polyphenols in an Elderly Mediterranean Population. Nutrients 11. https://doi.org/10.3390/nu11092165

Guan, H., Wang, M., Li, X., Piao, F., Li, Q., Xu, L., Kitamura, F., Yokoyama, K., 2013. Manganese concentrations in maternal and umbilical cord blood: Related to birth size and environmental factors. Eur. J. Public Health 24, 150–157. https://doi.org/10.1093/eurpub/ckt033

Institute of Medicine (US) Panel on Institute of Medicine (US) Panel on Micronutrients, 2001. Institute of Medicine (US) Panel on Micronutrients. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. National Academies Press (US), Washington, DC.

Krachler, M., Rossipal, E., Micetic-Turk, D., 1999. Trace element transfer from the mother to the newborn - Investigations on triplets of colostrum, maternal and umbilical cord sera. Eur. J. Clin. Nutr. 53, 486–494. https://doi.org/10.1038/sj.ejcn.1600781

Palau Miguel, M., Ligia Guevara Alemany, E., Moreno Seisdedos, M., 2008. Calidad de agua de consumo humano en España. Informe técnico. Trienio 2005-2006-2007. Madrid.

Rubio, C., Gutiérrez, Á.J., Revert, C., Reguera, J.I., Burgos, A., Hardisson, A., 2009. Daily dietary intake of iron , copper , zinc and manganese in a Spanish population. Int. J. Food Sci. Nutr. 60, 590–600. https://doi.org/10.3109/09637480802039822

Sánchez, C., López-jurado, M., Aranda, P., Llopis, J., 2010. Plasma levels of copper , manganese and selenium in an adult population in southern Spain : Influence of age , obesity and lifestyle factors. Sci. Total Environ. 408, 1014–1020. https://doi.org/10.1016/j.scitotenv.2009.11.041

Spencer, A., 1999. Whole Blood Manganese Levels in Pregnancy and the Neonate. Nutrition 15, 731–734.

Takser, L., Lafond, A.J., Bouchard, M., St-amour, G., Mergler, D., 2004. Manganese levels during pregnancy and at birth : relation to environmental factors and smoking in a Southwest Quebec population. Environ. Res. 95, 119–125. https://doi.org/10.1016/j.envres.2003.11.002

World Health Organization, 1996. Trace elements in human nutrition and health World Health Organization. World Health Organization, Geneva.

Zheng, W., Fu, S.X., Dydak, U., Cowan, D.M., 2011. Biomarkers of Manganese Intoxication. Neurotoxicology 32, 1–8. https://doi.org/10.1016/j.neuro.2010.10.002.Biomarkers

Zota, A.R., Ettinger, A.S., Bouchard, M., Amarasiriwardena, C.J., Schwartz, J., Hu, H., Wright, R.O., 2008. Maternal Blood Manganese Levels and Infant Birth Weight Ami. Epidemiology 20, 1–7. https://doi.org/10.1097/EDE.0b013e31819b93c0

ARTÍCULO II

Title: Urinary arsenic species and methylation efficiency during pregnancy: concentrations and associated factors in Spanish pregnant women

Reviewers' comments

Reviewer #1

This study by Soler-Blasco et al. investigated dietary and demographic predictors of speciated urinary arsenic in a cohort of pregnant women in Spain. This is an important topic which merits investigation, and the large sample size is a major strength. However, I do have some concerns about the presentation of the results and the authors' interpretation of the results, which are outlined below.

We thank the reviewer for all his/her suggestions and the recommended references. The authors have tried to improve the manuscript following the reviewers suggested. The specific points raised by the reviewer are addressed below. We have tracked changes in the text.

Highlights

"Tobacco consumption and body mass index were related with the As methylation". It would be nice to see this split into 2 different highlights which each provide more detail about the specific associations observed.

As the reviewer has suggested, we have separated this highlight in two in order to provide specific details about the relationships between these variables and the As metabolism.

Highlights

- *Tobacco consumption was associated with worse As methylation.*
- *Higher body mass index was related to better As methylation.*

Abstract

Background

The authors state that diet is the main source of exposure to arsenic. However, this is very population-dependent. Please consider rewording to acknowledge that

drinking water is also a major source of exposure for many populations.

We have added this information to the abstract.

- Arsenic (As) is considered to be toxic for humans, the main routes of exposure being through drinking water and diet.

"This element and its compounds are biotransformed in the body producing different metabolites". This wording is a bit awkward. Please consider rewording to "once ingested, inorganic arsenic can be methylated sequentially to monomethyl and dimethyl arsenicals" or something similar.

As the reviewer has suggested, we have reworded this sentence in the abstract.

As currently worded, the objectives presented in the Abstract do not add much to the current literature. The concentrations of different arsenic species and their associated factors have previously been described for pregnant populations (e.g., PMID: 32719440, 31690343, 28553665, 30068932, 14644662, 21078382). It would be helpful if the authors were more specific about what this particular study adds to the existing literature.

In the objectives, we have added the specific population where the study was carried out: *"Objectives: To describe the urinary concentrations of the different As species and evaluate the methylation capacity during pregnancy, as well as their associated factors in a birth cohort of Spanish pregnant women".*

Currently, little is known about prenatal arsenic exposure and As metabolism in low-exposure areas, such as Spain. The authors of the study consider that the present study can contribute to improve the knowledge about 1) As concentrations and its metabolites in a large population with high consumption of seafood and rice and, also, the factors associated to these urinary levels; 2) the As metabolism efficiency, evaluated through two approaches (in order to improve the comparability with previous studies), and the factors associated with the metabolism efficiency (including the OCM nutrients estimates and other elements).

Results:

Does total arsenic refer to the sum of iAs, MMA, and DMA or to the sum of iAs, MMA, DMA, and AsB?

Total As has been determined by a different analytical method to that employed with the species, as is explained in the methods section. This concentration was determined

by introducing the whole (diluted) sample into the ICPMS, without separation by HPLC (high performance liquid chromatography). Therefore, it has not been calculated for the sum of the species.

Do rice and seafood consumption refer to any consumption during the pregnancy? Or over the past year? Or to specific quantities or frequencies? Please be more specific.

We have added more specific information about dietary variables, both in the abstract and in the manuscript.

- Abstract, Results: "*Daily consumption of rice and seafood during the first trimester of pregnancy was positively associated with the concentration of As species*".
- Material and methods section, Covariates, Dietary variables (page 2): "*Information on diet during the first trimester of pregnancy was obtained from a 100- item semi-quantitative food frequency questionnaire (FFQ) completed at the time of sampling. The dietary information covered the time from the last menstruation to the first prenatal visit that occurred between weeks 10 and 13 of pregnancy. This FFQ was validated with good reproducibility for nutrient and food intake (Vioque et al., 2013). The items had nine possible responses, ranging from 'never or less than once per month' to 'six or more per day'. A commonly used serving size was specified for each food item in the FFQ. This was converted to average daily intake in grams for each individual participant*".

Please be specific about which arsenic metabolites were associated with vegetable and legume intake. Also, please specify if you are referring to "ever intake" during pregnancy or some other measure of intake.

We have added information about the As species associated with vegetables and legumes consumption. Also, in the previous sentence where the consumption of rice and fish is mentioned, reference is made to the frequency of food consumption, as well as the period when they were evaluated (it refers to all the food groups studied):"*Daily consumption of rice and seafood during the first trimester of pregnancy were positively associated with the concentration of As species (i.e., β [CI95%]= 0.36 [0.09, 0.64] for rice and iAs, and 1.06 [0.68, 1.44] for seafood and AB). TAs, AB and iAs concentrations, and DMA and MMA concentrations were associated with legumes and vegetables consumption, respectively". ~~The intake of vegetables and legumes was also associated with some species.~~".*

Please also provide relevant effect estimates/test statistics for results presented in the abstract.

We have added a couple of effect estimates coefficients: *"Daily consumption of rice and seafood during the first trimester of pregnancy were positively associated with the concentration of As species (i.e., β [CI95%]= 0.36 [0.09, 0.64] for rice and iAs, and 1.06 [0.68, 1.44] for seafood and AB)"* .

How are you defining higher methylation efficiency?

We have added the following information about the definition of higher methylation efficiency:

- Abstract, Results section: *"Non-smoker women and those with higher body mass index presented a higher ~~The consumption of tobacco and the BMI were also associated with the~~ methylation efficiency (denoted by a higher %DMA and lower % MMA)".*

Discussion:

Consider rewording *"the rice and seafood consumption were the major contributors to…"* to something such as "of the variables considered, rice and seafood consumption were the largest contributors to…". Also, what is this based on? R-squared? The magnitude of the effect estimate? Or another measure? This should be stated here.

The contribution of each variable to the As concentrations was based on the regression beta coefficient and 95%CI (higher effect estimate coefficients). We have added some of these effect estimates coefficients in order to facilitate the interpretation of the results shown in the abstract. Also, considering the reviewer's suggestion and another reviewer's comment, the discussion section of the abstract has been changed, in accordance with a suggestion made by another reviewer.

More details about the associations for tobacco smoke and BMI are needed. Were these variables associated with As methylation profiles that are indicative of increased or decreased metabolism?

We have added this information: "*Non-smokers women and those with higher body mass index presented a higher ~~The consumption of tobacco and the BMI were also associated with the~~ methylation efficiency*".

"Further birth cohort studies in low exposure areas are necessary to improve knowledge

about the arsenic exposure and its potential health impact". Several pregnancy birth cohorts studying arsenic in low-exposed populations already exist. Please be more specific about the types of studies that are needed.

We have added some information about the type of studies that the authors think that are necessary: "*Further birth cohort studies in low exposure areas are necessary to improve knowledge about arsenic exposure, especially of inorganic forms, and its potential health impact during childhood*".

Introduction

The introduction is very long. I'm not sure that the first 2 paragraphs are needed.

Following the reviewer's advice, we have tried to abbreviate the introduction, by merging the information in the first two paragraphs into a single more abbreviated one.

"In certain regions, where the As concentrations in soil are naturally elevated, such as in Bangladesh, China, Taiwan, India, Argentina, Chile, México, Vietnam, Australia and USA, the main exposure route for inorganic forms of this element is through drinking water". As worded, it sounds like the authors imply that drinking water is the main source of exposure to arsenic for these countries, but that is not universally true for all of the countries listed.

We appreciate the reviewer's comment. We have reworded this paragraph in order to improve the explanation about the main routes of exposure of iAs.

Introduction section, (page 5): "*The main route of exposure to inorganic forms of As is through diet. In some areas of Bangladesh, India, Vietnam, China, Argentina, Chile, México, Australia and USA, the As levels in ~~groundwater~~ drinking water are above the maximum guideline value recommended by the World Health Organization (WHO) in 2003 (10 µg/L) (World Health Organization, 2017), water consumption being the main exposure route for iAs. In regions with low As levels in water, such as Spain, the main source of exposure to inorganic As (iAs) is rice (European Food Safety Authority, 2014)".*

"In regions with low As levels in water, such Spain, the main exposure route is through the diet". Please provide a supporting reference for this statement.

We have reworded this sentence: "*In regions with low As levels in water, such as Spain, the main source of exposure to inorganic As (iAs) is rice consumption (European Food Safety Authority, 2014)".*

"Some nutritional factors seem to increase the efficiency of iAs methylation, especially micronutrients involved in the one-carbon metabolism (OCM), such as zinc (Zn), B_6 and B_{12} vitamins and folic acid (Kurzius-Spencer et al., 2017; Laine et al., 2018)." Zinc is not a OCM nutrient and should be listed separately. Also, there have now been randomized clinical trials supporting the above statement for folate, which merit reference (PMID: 17093162, 30590411). There is also evidence that betaine and choline (also involved in the OCM pathway) influence arsenic metabolism (PMID: 32918135, 30590411). These nutrients may also be important and should be referenced here.

We thank the reviewer for the suggestions. We have separated Zn from the description of OCM nutrients. We have also referenced choline and betaine as OCM nutrients, adding the following reference (Heck et al., 2007). Finally, we have added the references of the randomized clinical trials which evaluate the effectiveness of folate supplementation in As metabolism.

- Heck JE, Gamble M V., Chen Y, Graziano JH, Slavkovich V, Parvez F, et al. Consumption of folate-related nutrients and metabolism of arsenic in Bangladesh. Am J Clin Nutr. 2007;85(5):1367–74.

"Arsenic absorbed by the gastrointestinal tract is biotransfomed mainly in the liver". Please provide a supporting reference.

We have added a supporting reference:

- Drobná, Z., Walton, F. S., Paul, D. S., Xing, W., Thomas, D. J., & Stýblo, M. (2010). Metabolism of arsenic in human liver: The role of membrane transporters. Archives of Toxicology, 84(1), 3–16.

"Regarding organic forms, AB is excreted unchanged in urine, but the biotransformation of arsenosugars or lipid soluble As species is less well understood (European Food Safety Authority, 2009). The supporting reference is old. Please consider using an updated reference. There is evidence that arsenosugars and arsenolipids are biotransformed to dimethyl arsenic.

We have added the information proposed by the reviewer and some recent references about the metabolism of arsenosugars and arsenolipids.

Introduction section, (page 6): *"Regarding organic forms, AB is excreted unchanged in urine, but other organic forms, such as ~~the biotransformation of~~ arsenosugar and arsenolipid ~~lipid soluble As~~ species present in seafood, seem to be metabolized producing DMA ~~is less well understood~~* (European Food Safety Authority, 2009; Molin et al., 2012; Taylor et al., 2017)".

"In this scheme, ingested iAs is reduced (from arsenate -iAs$_V$- to arsenite -iAs$_{III}$-) and methylated, to monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA), being S-adenosylmethionine (SAM), the main donor of methyl group (Cullen, 2014)." There is also human evidence supporting the role of SAM as a methyl donor for As metabolism (PMID: 24598884)

We appreciate the reviewer's suggestion. We have added the reference PMID: 24598884 to support this sentence.

"After this biotransformation process, iAs is excreted through the urinary system as DMA (60-80%), MMA (10-20%) and iAs (10-30%)." Please provide a supporting reference.

We have added the following reference to support the statement:

- Vahter ME. Variation in human metabolism of Arsenic. In: Chappell W, Abernathy C, Calderon R, editors. Arsenic exposure and health effects. Oxford: Elsevier Science; 1999.

"The relative concentrations of each metabolite reflect the individual iAs methylation capacity". This is not necessarily true. The metabolite concentrations may also reflect dietary sources, especially in populations exposed to low levels of As through drinking water.

We have clarified this sentence.

Introduction section (page 5): "*These relative concentrations ~~of each metabolite~~ reflect the iAs methylation efficiency, indicated by a high %DMA and lower %MMA and %iAs, especially in populations exposed to high levels of iAs through drinking water*–(M. E. Vahter, 1999)*".*

We have not added any more information about the dietary source of the As metabolites in an attempt to keep the introduction section as concise as possible. However, we have explained in the discussion section how the consumption of some foods, such as fish, can also influence the concentrations of certain metabolites.

"Therefore, the aim of this study is to describe the concentrations of total As (TAs) and the different urinary As species (DMA, MMA, AB and iAs) and the methylation capacity during pregnancy in Spain." Demographic and dietary predictors of arsenic and arsenic metabolism have been examined in other pregnant populations (e.g., PMID: 32719440, 32007748). It may be worth explaining why it is important to address this question in this particular population.

The authors consider that this study may contribute to the literature with some important questions. Firstly, as far as we know, this is the European study with the largest sample size up to now that has analysed the different As species concentrations in urine and the As metabolism efficiency in pregnant women. We believe that this information is relevant, because

the As exposure has not been extensively studied in areas with low As exposure through water, such as in Spain. Further knowledge about the sources of exposure to As, especially for the inorganic forms, in certain populations, could help to develop dietary recommendations for pregnant women in these areas in order to avoid the possible deleterious effects.

We have modified the objective of the study by including the country where it was conducted and we have added more information about the importance of the results in the discussion:

Discussion section (page 22): "*The major strength of the present study is the analysis of As speciation in a considerably large sample size. In fact, as far as we know this is the largest European study describing As species concentrations and methylation efficiency in pregnant women*".

"Some nutritional factors seem to increase the efficiency of iAs methylation, especially micronutrients involved in the one carbon metabolism (OCM), such as zinc (Zn), B6 and B12 vitamins and folic acid (Kurzius-Spencer et al., 2017; Laine et al., 2018)". As mentioned above, Zn is not part of the OCM pathway. Please reword. Also, the evidence for folic acid is very strong and supported by RCTs in addition to a large amount of observational data, which should be referenced here. The evidence for B12 is indeed mixed, but few of those supporting studies are included here. And for B6, several observational studies have now supported a possible association between this nutrient and arsenic metabolism, beyond the one study (Kurzius-Spencer) that is listed here (e.g. please see PMIDs: 29070711, 31913474, 28479390).

We have separated Zn from the description of OCM nutrients, and we have added the references of the randomized clinical trials that evaluate the effectiveness of folate supplementation in As metabolism.

We have also added the references suggested by the reviewer that support the influence of vitamins B_6 and B_{12} in As metabolism.

Our most sincere thanks go to the reviewer for suggesting all these references that are helping to notably improve this work.

Methods:

The authors note that the main reason for non-participation in the current study was the lack of arsenic speciation measures due to limited funding. How were participants selected for these measures? Was it solely based on sample availability? Or were there other selection criteria? These details should be included.

The urinary samples of the pregnant women were selected taking into account that we had longitudinal information about their children until 2 years old. We followed this selection criterion in order to assess the potential health effects during childhood related to prenatal As exposure in further works. Among the total samples that met this criterion, we randomly selected a smaller number of samples, due to the limited funding available for this project.

We have added this information in the manuscript.

Materials and methods section, Study population (page 7): "*The final study population was made up of 1017 mothers with available urinary arsenic species concentrations at first trimester of pregnancy (69.4% of total recruited participants). These mothers were selected taking into account two criteria: 1) availability of longitudinal information about their children until 2 years-old (in order to assess the potential health effects of prenatal As exposure in further studies), and 2) among the women who met the first criterion we randomly selected a subsample of 1017 due to limited funding for the As analysis. ~~main reason of non-participation was the unavailability of funding to measure the As speciation in all samples~~*".

"Different analyses were performed to detect any differences between the included and nonincluded population". This is very vague. What analyses were performed?

We have now added information about the analyses performed in the text.

Materials and methods section, Statistical analysis (page 10): *"Descriptive and bivariate analyses using Fisher's Exact Test for categorical variables and Kruskall Wallis Test for continuous variables were performed in order to detect any differences between the included and the excluded populations".*

Were any duplicate samples sent out for arsenic speciation analyses? If so, what were the %CVs?

No duplicate samples were sent out for arsenic speciation analyses. However, as a quality control, 102 of the 1017 samples analysed were re-analysed, which represents 10%. These second measurements matched the first measurement on average in 100 ± 3 cases %.

"When the difference was larger than 15%, either the total arsenic or the speciation analysis was repeated". What number and % of samples needed to be repeated?

The number of samples that presented this variation, and for which the analysis was repeated, was 102 (10%).

For the BMI categories presented, did the "low healthy weight" category include women who were underweight (BMI<18.5)? If so, perhaps reword this category.

We thank the reviewer for noticing this mistake. We have reworded the category in order to avoid misunderstandings.

Materials and methods section, Sociodemographic variables (page 8): "*body mass index (BMI, kg/m^2) before pregnancy (continuous and categorized by low and healthy weight [<25], overweight [25-<30], obesity [≥30])".*

"Additionally, dietary folate, folic acid, B_{12} and B_6 vitamins, iron (Fe) and Zn intake were estimated using the food composition tables of the US Department of Agriculture (U.S. Department of Agriculture: Agricultural Research Service USDA, 2007) and with Spanish sources (Palma et al., 2008)." Could choline and betaine intakes also be calculated? These nutrients are important methyl donors that are also involved in the OCM pathway, and there is evidence that they may influence arsenic metabolism (PMIDs: 27565879, 32918135)

Unfortunately, we do not have the estimated choline and betaine intakes for the pregnant women of the INMA cohort. We have added this fact as a limitation of our study:

Discussion section (page 22): "*This study has several limitations (…) 3) the present study lacks information on some important nutrients involved in OCM and related to As metabolism, such as choline and betaine, and so the influence of these variables could not be tested*"

Was Spanish the primary language of all participants? If not, were questionnaires offered in other languages if needed?

In the two areas used in the study, the official language was Spanish. Nevertheless, in the Gipuzkoa cohort, the questionnaires were also offered in the co-official language (Basque).

I would recommend the use of directed acyclic graphs or other hypothesis-driven approaches to inform covariate selection for statistical models, rather than solely relying on data-driven approaches.

We thank the reviewer for this suggestion. We have not used the directed acyclic graphs for the selection covariates, but our statistical method is not only based on data-driven approaches. In fact, the first step to select the variables that could be related to the As concentrations was a thorough literature review about this topic. Then, the potential variables were selected through the statistical analysis described in the manuscript.

We have added this specific information in the Covariates section (Study variables and sources of information). Moreover, we have included some references about factors related to As

exposure and metabolism (page 8): "*We selected the covariates used in this study from the previous literature on this topic* (European Food Safety Authority, 2014; Nigra et al., 2019; Saxena et al., 2018; Shen et al., 2016; C.-H. Tseng, 2008)".

"Although food intake variables were mutually correlated (Figure S1), we found no collinearity among them". How was collinearity evaluated? It would be nice to see a similar correlation plot in the supplement for the nutrients that were examined.

Collinearity was evaluated with the Variance Inflation Factors in the final models. We have added a bit more information about how collinearity was evaluated in the last paragraph of the Statistical Analysis section.

Material and methods section, Statistical Analysis (page 12): "*Variance inflation factors (VIFs) were used as a check for collinearity among variables in the final models, all VIFs being <2.5*".

Moreover, as the reviewer has suggested, we have added a correlation plot for estimated nutrients in the supplementary material (Figure S2).

Was specific gravity measured? Can you compare results adjusting for specific gravity instead of urinary creatinine? Urinary creatinine is related to muscle mass, sex, and diet, and has itself been associated with arsenic metabolism (PMID: 16002357). Correcting arsenic measures for creatinine may therefore complicate interpretation of the results.

Unfortunately, we have not information about specific gravity. In the present analysis we have used the approach proposed by Barr et. al, including creatinine concentrations in multivariate models of the association between As species concentrations and the related factors as a separate independent variable (Barr et al., 2005). When the dependent variables were the percentages of As species, the As metabolites were calibrated by arsenobetaine and adjusted for creatinine concentrations by regressing As metabolites against creatinine and arsenobetaine. In the discussion section we have added the limitation of using creatinine concentrations to control for urinary dilution:

Discussion section (page 22): "*This study has several limitations (…) 4) finally, we used creatinine concentrations to control for urinary dilution. Creatinine concentrations seem to be associated with As metabolism. To control for this effect, we used the approach proposed by Barr et. Al (2005), including the creatinine concentrations in the multivariate models as a separate independent variable. Nevertheless, due to the complexity of the interrelations between As metabolism, micronutrients and creatinine, the interpretation of the factors associated with As metabolism should be taken with caution*".

"The validity of the regression models was tested by residual analyses". This is a bit confusing. Do the authors mean something like the following?: "To check whether linear regression assumptions were met, we visually inspected model residuals for normality and homoscedasticity".

We have modified this sentence following the reviewer's suggestion.

Results:

"Consumption of fish showed a clear increasing trend with AB and TAs concentrations" Ever vs. never consumption? Or was this evaluated differently?

In order to clarify this point, we have added the following information about this variable.

Results section (page 13): "*Consumption of fish (< 1 serving per week, 1 serving per week, > 1 serving per week) showed a clear increasing trend with AB and TAs concentrations*".

"Consumption of more than 1 serving per week of rice increased DMA, MMA and iAs concentrations". As worded, this implies causality.

We have reworded this sentence following the reviewer's suggestion.

Results section section (page 13): "*Consumption of more than 1 serving per week of rice was associated with an increase in DMA, MMA and iAs concentrations*".

"Regarding the association with micronutrients involved in one-carbon metabolism, only the estimation of Zn daily intake was positively associated with %DMA". Zn is not an OCM nutrient.

We have reworded this sentence in order to separated Zn from the description of OCM nutrients in the results.

"No other elements, such as serum Se or Mn concentrations or estimated folate, B_6 and B_{12} vitamin intake were related with any As metabolite percentages (data not shown)". It would be nice to see these data. Please consider adding the results to the supplement.

We have added the bivariate analysis of the methylation efficiency (evaluated by calibrated percentage of As metabolites and the two principal components) and estimated nutrients intake (adjusted by calories) as well as essential and toxics elements in the supplementary material (Table S3).

Results section (page 15):

We have commented on the table in the **Results section (page 15):** "*Regarding the estimated nutrients, ~~the association with micronutrients involved in the one-carbon metabolism~~, only the estimation of daily intake of Zn was positively associated with %DMA in the bivariate analysis (Table S3), and this association continued to be present in the multivariate model, although in a marginal way (β [95%CI]: 7.71 [-1.39, 16.81]). The micronutrients involved in the one carbon metabolism evaluated (estimated folate and vitamins B_6 and B_{12} intake) and other elements evaluated (serum Se and Mn concentrations) were not related to As metabolite percentages in the bivariate or the multivariate analyses (Table S3)".*

It would be helpful to know the N (%) of participants who were not consuming sufficient quantities of the nutrients evaluated and also the N (%) of participants who were consuming nutrients above recommended intake levels if relevant (e.g., above UL for folate). Could these details be added to a supplemental table?

As the reviewer recommended, we have added information in Table S1 about the percentages of women who were below and above of the Dietary Reference Values for vitamins B_6 and B_{12}, folate, Fe and Zn, using the references values for pregnant women (the Population Reference Intake for Fe, Zn and vitamin B_6 and adequate intake for folate and vitamin B_{12}) of the European Food Safety Authority (European Food Safety Authority, 2019)(The National Academies of Sciences, Engineering, 2019)(The National Academies of Sciences, Engineering, 2019)(The National Academies of Sciences, Engineering, 2019)(The National Academies of Sciences, Engineering, 2019)(The National Academies of Sciences, Engineering, 2019)(The National Academies of Sciences, Engineering, 2019). We have also added information about the percentage of women with anaemia at the first trimester of pregnancy (serum ferritin lower than 15 µg/L) (Supplemental, Table S1).

It would also be interesting to know the N (%) of participants who were taking prenatal (or other) vitamins during the pregnancy. If a sufficient number of participants were not taking prenatal vitamins in early pregnancy, this would also be an interesting variable to examine in relation to the arsenic species.

We thank the reviewer for this suggestion. In our population, only 4% of the pregnant women did not take any kind of vitamins during the period under study. We consider that the number of participants who were not taking prenatal vitamins is too low to examine the association with As species.

Discussion:

The headers in the Discussion section seem unnecessary.

We thank the reviewer for this comment but we would prefer to keep the subheadings in this section because we consider that they are useful for understanding and following of the discussion.

"However, we observed that As and its metabolites concentrations were slightly higher than those observed in pregnant populations from regions with low As levels in water". Please provide a supporting reference for this statement.

Following the reviewer's advice, we have moved this sentence to the section containing the comparison with the results observed in the previous studies together with the respective references.

Discussion section (page 16): "*In our study, iAs and MMA urinary concentrations were higher than in other studies carried out on regions with low levels of iAs in drinking water, such as some areas of USA, Croatia, Slovenia and Canada (Howe et al., 2020; Stajnko et al., 2019; Vaughan Watson et al., 2020). Conversely, in our study urinary AB concentrations were higher than in other studies conducted in USA, the main reason probably being the high fish and seafood consumption of the Spanish population*".

"A possible explanation seems to be related to the nutrients involved in the OCM (folate and vitamin B_{12}, Zn, Fe, choline and methionine), which are closely related with a better metabolism and excretion of As (Kurzius-Spencer et al., 2017)." Zn and Fe are not OCM nutrients. And folate is not found in red meat. It is most abundant in leafy greens (or fortified foods or supplements). Perhaps reword to "These associations may reflect nutrients found in red meat that have been associated with As metabolism, such as Zn and Fe, and certain OCM nutrients, such as B12, choline, and methionine".

As a reviewer has suggested, we have reworded this sentence.

"A study carried out in USA showed a slightly better methylation among Hispanic people than non-Hispanic white, African American and Chinese American people (Balakrishnan et al., 2018)." A recently published study on another pregnancy cohort in the U.S. also reported evidence of better methylation capacity among Hispanic compared with non-Hispanic women (PMID: 32719440).

We thank the reviewer for suggesting this reference. We have added the references and commented on it in the discussion section. We have also added this study to Table 4.

Discussion section (page 19): *"A study carried out in USA showed a slightly better methylation among Hispanic people than non-Hispanic white, African American and Chinese American people (Balakrishnan et al., 2018). In the same way, a recent study has reported a better methylation pattern (denoted by higher %DMA and lower %iAs) in US-born and foreign-born pregnant Hispanic women, compared with non-Hispanic ones (Farzan et al., 2020)".*

"We did not observe any statistically significant association between As methylation efficiency and the OCM nutrients concentrations (folate, vitamin B_{12} and B_6 or Zn) measured in our study."
My understanding was that dietary intake levels (not concentrations) were evaluated for most of these nutrients.–These null findings are also very interesting and merit some additional discussion, especially in the context of the many other studies (both RCTs and observational) that have observed evidence that OCM nutrients influence As metabolism (e.g., PMIDs: 30590411, 17093162, 24598884, 29070711, 31913474). What are the potential reasons for the discrepancies? For example, across 4 different studies and populations (PMIDs: 29070711, 31913474, 28697391, 28479390) vitamin B6 has been associated with enhanced As metabolism (although none of these previous studies were conducted in pregnant populations). It would be interesting to hear some hypotheses from the authors about why B6 was not associated with As metabolism in this Spanish cohort. Could it be due to the B6 levels in this population, differences due to pregnancy status, or other population differences? There is also quite a bit of evidence linking folate to As metabolism (both from RCTs and observational studies), especially in populations with a high prevalence of folate deficiency (PMID: 30590411, 17093162, 24598884, 30068932). In contrast, null results have been observed in some studies of predominately folate-sufficient populations (PMID: 29070711, 16140620). This may be due to the fact that in some populations, dietary folate intake is in excess of recommended intake levels due to the widespread use of supplements and the consumption of fortified foods (PMID: 29070711). The authors should provide some details about folate fortification in Spain and also supplement use in Spain (and among pregnant women more generally) to help improve the interpretation of these results. Some discussion about how baseline nutritional status can impact nutrient-As metabolism relationships may also be helpful for interpreting these results (PMIDs: 24598884, 32918135). Additionally (as noted above), Zn is not an OCM nutrient and should be removed from this sentence. The Zn findings merit their own section in the Discussion.

We thank the reviewer for his/her suggestions about the discussion of the OCM nutrients and As metabolism. Because the next three comments are related to this topic, we have answered them here.

As one reviewer has suggested, we have added more information about the lack of relationship between OCM nutrients and the As metabolism. We have now explained this point better in the discussion.

Discussion section (page 21): "*Previous studies observed an influence of some of these nutrients on iAs methylation, such as folate and vitamin B_6 and B_{12} vitamins, which are involved in the synthesis of S-adenosylmethionine (SAM), the main donor of methyl group in the iAs methylation* (Bozack et al., 2019; Gamble et al., 2005; Howe et al., 2017; Kurzius-Spencer et al., 2017). *However, the results for studies conducted on pregnant women populations have been heterogeneous; thus, in a Mexican cohort, no association was observed between serum B_{12} vitamin levels and the percentages of the urinary arsenic metabolites (Laine et al., 2018). A Bangladeshi pregnant women study showed a marginal negative association with plasma folate and %iAs, but no association with B_{12} vitamin was found (Li et al., 2008). Additionally, plasma folate was inversely associated with the urinary percentage of As^{+5} before delivery (Hall et al., 2007). However, in that same cohort, an increase in methylation capacity through the pregnancy was observed regardless of the women's folate and vitamin B_{12} status (Gardner et al., 2011). It has been suggested that As metabolism is more efficient during pregnancy due to an increase in the endogenous synthesis of the methyl-donor choline, to supply the high fetal demand ~~more efficient maternal one-carbon metabolism in order to supply the high fetal demand~~* (M. Vahter, 2009). *This specific process during pregnancy may lead to certain cofactors or methyl-donors, such as folate, having a marginal influence on As metabolism (Gardner et al., 2011). Moreover, it is possible that in well-nourished populations it is more difficult to observe the influence of the one-carbon nutrients than in populations with nutritional deficiencies. In fact, Howe (2014) showed a positive association between blood SAM and %MMA in folate- and cobalamin-deficient participants, but this association was not found in the micronutrient-sufficient group. In our populations, only 22%, 2% and 16% of women had estimated levels of vitamins B_6, B_{12} and folate intakes, respectively, below the recommendations (European Food Safety Authority, 2019), and only 4% of the participants did not take folic acid supplements at the first trimester of pregnancy".*

We have also added a new paragraph to discuss the Zn results.

Discussion section (page 21): "*We also observed an association between the estimation of Fe intake with the As metabolism efficiency, specifically with decreasing PC1. In experimental studies, this element seems to diminish the bioaccessibility of As in the gut (Yu et al., 2016). Nevertheless, in a randomized controlled trial study conducted with Mexican children, the supplementation with Fe had no impact in the As metabolism (Kordas et al., 2017). In this same*

study, Zn supplementation was not related to better As metabolism. These results seem to be in disagreement with those found in our study, where estimated Zn consumption was positively associated with the %DMA, although the coefficients did not reach statistical significance. Similarly to our findings, in an observational study conducted on Mexican women, the estimated Zn intake was related to a decrease in %MMA and %iAs, and an increase in %DMA (López-Carrillo et al., 2016). Zn is a necessary cofactor of betaine homocysteine methyltransferase (BHMT; EC 2.1.1.5). This enzyme uses betaine as a methyl donor and Zn as a cofactor for the remethylation of homocysteine to methionine (Millian & Garrow, 1998)*".*

"In addition, it has been suggested that the As metabolism is increased through pregnancy due to a more efficient maternal one-carbon metabolism in order to supply the high fetal demand (Vahter, 2009)." One-carbon metabolism is a biochemical pathway, so this is worded a bit strangely. It has been hypothesized that an increase in choline availability occurs during pregnancy (due to increased PEMT activity) which is thought to be responsible for the apparent increase in As methylation capacity.

We have answered this suggestion in the previous comment.

"This could be the reason of the inconsistency in the results related to OCM nutrients." Some studies of pregnant women have observed associations between OCM nutrients and arsenic metabolism (e.g., 17938743), so I'm not convinced that pregnancy status completely explains the null results here. Another potential explanation, which was not discussed, is population differences in nutritional status. How did dietary intake levels for these particular nutrients compare with other studies that evaluated nutrient-arsenic metabolism associations?

We have answered this suggestion in the previous comment.

"This study has several limitations. First, around 30% of the recruited participants were not included in this study. This lack of participation was due to the unavailability of funding to measure the As speciation in all urine samples." How were participants selected for speciated urinary As measures? Were they randomly selected among all who provided urine? If not, how might this have impacted the results?

We have answered this suggestion in the next comment.

"Nevertheless, we did not find substantial differences on the characteristics between the participant and no-participant populations" Please be more specific. What do you mean by substantial differences? And how might those differences influence the results?

As the reviewer previously suggested, we have added more information about the selection of the participants.

Materials and methods section, study population (page 7): "*The final study population was made up of 1017 mothers with available urinary arsenic species concentrations at first trimester of pregnancy (69.4% of total recruited participants). These mothers were selected taking into account two criteria: 1) availability in longitudinal information about their children until 2 years-old (in order to assess in further studies, the potential health effects of prenatal As exposure), 2) among the women who met the first criteria we randomly selected a subsample of 1017 due to limited funding for the As analysis*".

We have also reworded this point in the limitations paragraph, avoiding the term "any substantial differences" and trying to explained better the potential influence of the differences between the included and excluded population (page 22): "*This study has several limitations: 1) around 30% of the recruited participants were not included in this study and participants could have a more privileged socioeconomic profile than non-participants. This fact could be related to dietary habits that can lead to differences in exposure to different forms of As*. ~~Another~~ ~~This lack of participation was due to the unavailability of funding to measure the As speciation in all urine samples. Nevertheless~~, ~~we did not find substantial differences on the characteristics between the participant and no-participant populations~~".

"Another limitation in our study could be related to the assessment of the As exposure at only one time point during pregnancy. This could reflect only recent As exposure and it could be no representative of overall pregnancy period." What was the range for the gestational age at urine collection? Was gestational age at urine collection examined as a possible predictor of urinary As or the As metabolism indices? Given evidence that As metabolism changes during pregnancy (PMID: 21078382, 14644662), this is worth examining.

We thank the reviewer for his/her suggestion. The median gestational age at sampling (percentile 25,75) was 12.7 (12.3, 13.4) weeks of gestation.

We have performed a bivariate analysis between the gestational age variable and each outcome variable (all of them adjusted for area of study and creatinine). Gestational age was only associated with MMA concentrations and %MMA (p value <0.2). We have tested gestational age in the multivariate models of MMA concentrations and the %MMA and it was retained in the models. We have updated these new models in the results section, Figure 1 and Table 3.

Results section (page 15): *"Furthermore, gestational age at sampling was inversely related to %MMA (β [95%CI]: -0.84 [-1.32, -0.36])".*

Discussion section (page 20): *"The gestational age at sampling was inversely related to the %MMA. This result is consistent with previous studies, which show an increase in methylation capacity throughout pregnancy, denoted by higher %DMA and lower %MMA and iAs (Gardner et al., 2011; Hopenhayn et al., 2003). It has been suggested that the As metabolism is increased during the course of pregnancy due to a more efficient maternal one-carbon metabolism that increases the endogenous synthesis of the methyl-donor choline, to supply the high fetal demand for correct development* (M. Vahter, 2009). *Nevertheless, in our study, gestational age was only related to %MMA, but it was not associated with an increase in %DMA. Unfortunately, in our study we have measured As metabolites concentrations in one-spot urine samples during pregnancy, which prevents us from evaluating whether this decreasing trend in %MMA is due to an improvement in As methylation or a change in the maternal diet".*

"Another advantage is the analysis of As methylation efficiency through two approaches, allowing comparability of results." This is a bit vague. For clarity, please explain what two approaches you are referring to and how they differ.

We have added additional information about the advantage of evaluating the efficiency of As methylation through two approaches.

Discussion section (page 22): "*Another advantage is the analysis of As methylation efficiency through two approaches: using the relative percentages of each As metabolite and, in order to minimize the high correlation between the three percentages, using a principal component analysis. This allows comparability with previous studies that have used either of these two methods to evaluate the efficiency of arsenic metabolism*".

Reviewer #2:

The paper by Soler-Blasco et al. details the associations between several sociodemographic and dietary factors and methylation of inorganic arsenic, and concentrations of other arsenic species among pregnant women. The study is among the participants of the INMA cohort and has rich data on several variables. There are some points that the authors need to clarify in order to strengthen the manuscript.

We are very grateful for the reviewers' careful reading of our manuscript, which has allowed us to clarify some aspects of it. The specific points raised by the reviewers are addressed below. We have tracked changes in the text.

Abstract:

Background - please specify that the biotransformation in the body occurs mainly through methylation reactions.

We have changed the sentence to add the information proposed by the reviewer:

Abstract, introduction: *"Once ingested, inorganic arsenic can be methylated sequentially to monomethyl and dimethyl arsenicals"*.

Discussion: Lines 43-47 are results. So, as part of the discussion, instead of stating those results, please include comments on what those results signify.
The abstract should specify the study design.

In accordance with to the reviewer's suggestion, we have slightly changed the discussion section of the abstract. We have removed the specific results from this section: "***Discussion****: Certain dietary, lifestyle, and environmental factors were observed to have influence on both As species concentrations and methylation efficiency in our population. ~~Among the variables considered, rice and seafood consumption were the largest contributors to The rice and seafood consumption were the major contributors to the urinary As concentrations species during pregnancy in our population. The consumption of tobacco and the BMI were also associated with higher methylation efficiency.~~ Further birth cohort studies in low exposure areas are necessary to improve knowledge about arsenic exposure, especially of inorganic forms, and its potential health impact during childhood"*.

We have also added the study design in the methodology section: "*Participants in this cross-sectional study ~~Study subjects~~ were 1017 pregnant women from two Spanish areas participants in the INMA (Environment and Childhood) project (2003-2008)".*

Introduction

Line 39: Please replace "nowadays" with "currently".

As the reviewer has suggested, we have replaced the word "Nowadays" by "Currently" in the manuscript.

The third paragraph of the introduction needs to include details about the general proportions of urinary iAs metabolites that would indicate efficient methylation, for example, low %iAs, low %MMA, and high %DMA is indicative of an efficient methylation.

We have added this information to the introduction section (page 5): "*These relative concentrations reflect the iAs methylation efficiency, indicated by a high %DMA and lower %MMA and %iAs, especially in populations exposed to high levels of iAs through drinking water* (M. E. Vahter, 1999)".

Line 52: The %DMA, %MMA, and %iAs given here are the typically observed proportions of these metabolites in the urine, not the proportions that may be observed in every individual's urine. The authors need to clarify this. Also, this fact needs a citation - Vahter, 1999, Methylation of inorganic arsenic in different mammalian species and population groups.

We have reworded this sentence in order to clarify the information. Furthermore, we have added the reference recommended by the reviewer:

Introduction section (page 5): "*After this biotransformation process, iAs is excreted through the urinary system. In general, the most frequent proportions of the metabolites observed in urine are 60-80% of DMA, 10-20% of MMA and 10-30% of iAs* (M. E. Vahter, 1999). *These relative concentrations ~~of each metabolite~~ reflect the iAs methylation efficiency, indicated by a high %DMA and lower %MMA and %iAs, especially in populations exposed to high levels of iAs through drinking water* –(M. E. Vahter, 1999)".

The adverse health effects of arsenic exposure are well described toward the end of the introduction section. However, the introduction should include/clarify further as to why

methylation efficiency matters. This can be borne out by detailing the findings about how poor methylation efficiency is shown to be a risk factor for arsenic induced adverse health outcomes.

We have added some information about adverse health outcomes are related to the methylation efficiency.

Introduction section (page 6): "*Furthermore, a lower As methylation capacity has been related to higher risk of skin lesions, bladder, lung and skin cancer, and peripheral vascular disease* (Gamboa-loira et al., 2017; C. H. Tseng, 2007)".

The first two paragraphs of the introduction can be tightened a bit, so that the introduction would still be concise after adding the part about poor methylation being a risk factor for outcomes.

As the reviewer has suggested, we have tried to reword the first two paragraphs in a more concise way.

The introduction should specify the design of the study.

We have added the study design in the methodology section.

Introduction section (page 7): "In this cross-sectional study, subjects were pregnant women participants in the INMA Project (Environment and Childhood)".

Methods

Study population - Lines 29-30 belong in the analysis section; please replace "non-included" with "excluded".

As the reviewer has suggested, we have replaced the term "non-included" with "excluded" in the manuscript and in the Table S1. We have also moved this part to the analysis section.

Lines 31-36 belong in the results section

As the reviewer has suggested, we have moved this part to the first paragraph of the results section.

Study variables and sources of information - Please add a section about creatinine measurement.

We have added some information about creatinine measurement and analysis:

Methods section, covariates (page 10): "*Creatinine concentrations were measured in the same urine samples at the first trimester of pregnancy by DRI® Creatinine-Detected® Test using AV680 from Beckman Coulter*".

Dietary variables - Line 29 - please replace "ailments" with "elements" or "foods and food groups"

We thank the reviewer for noticing this mistake. We have replaced the term "aliments" by "foods".

Statistical analysis- Line 35: Please clarify the meaning of "dietary variables" - does it mean foods, food groups, nutrients, or all of these? Or were all the factors mentioned in the "dietary variables" section included?

We have replaced "dietary variables" with "food groups" in order to clarify this sentence.

Just as a clarification question - was each dietary variable added to the model and then removed, followed by the addition of the next dietary variable?

Indeed, to build the final models, we added and removed each food group variable into the core models of sociodemographic and environmental variables one by one. Then, we added all the dietary variables at a level of $p<0.1$ in the likelihood ratio test into the core models.

Lines 42-43: Was urinary creatinine added to the model, in addition to the creatinine-adjusted urinary arsenic variable as the outcome? That would lead to concerns about over-adjustment of the model.

In the multivariate models, we used the urinary arsenic variables unadjusted by creatinine as the outcome (µg/L). We preferred to add the creatinine concentrations as an adjustment variable in the model.

Some previous publications from the INMA cohort have included the adjustment of urinary arsenic with urinary specific gravity. Was specific gravity not measured among participants of this study? Adjusting urinary arsenic for specific gravity has several advantages over creatinine adjustment.

As the reviewer has commented, there is a publication from the INMA cohort in which specific gravity was measured and it was used for the adjustment of urinary arsenic (PMID: 28553665).

Nevertheless, this measurement was only analysed in a subsample of 100 participants. Unfortunately, we did not have specific gravity measurements for the whole sample.

Results

Table 3: Please denote in the footnote that the dietary intakes were energy adjusted, and also denote that the models included all the presented variables simultaneously.

As the reviewer has suggested, we have added this information in the title and footnote of Table 3.

Discussion

Some nutrients are known to aid in the efficient methylation of inorganic arsenic. Conversely, some foods are considered sources of arsenic exposure. This study evaluated the associations of foods and nutrients with both, methylation efficiency as well as arsenic exposure. So, the discussion would be strengthened if the authors included a subsection about how to balance these - foods as sources of exposure vs. aiding in methylation efficiency. Assessing this is challenging methodologically, but based on the existing evidence, can a comment be made in regards to dietary guidelines for pregnant women? Tying the results and discussion back to dietary recommendations for pregnant women is particularly essential because pregnancy is a unique biological period; exposures happening during pregnancy affect the fetus as well as the maternal health.

We consider that the topic proposed by the reviewer is really interesting. In fact, the authors have discussed this subject in great depth. Indeed, there exist a tangled interrelation between the As exposure source (and its different species) and the nutrients that improve the As metabolism. Furthermore, the As metabolism is not yet fully understood, which makes it difficult to assess accurately. Finally, the literature about prenatal As exposure and the potential health effects during childhood are still very scarce in low-exposed areas, such as Spain.

For all these reasons, the authors consider that the potential dietary recommendations for pregnant women in a specific context, such as Spain (with low As concentrations in water and high rice and seafood consumption) should not be carried out until further epidemiological studies are conducted to improve our knowledge of these issues.

Also consider including the limitations associated with creatinine adjustment of urinary arsenic concentrations. N-guanidinoacetate methyltransferase, which is the main consumer of methyl groups from the human one carbon cycle, participates in the formation of creatinine from creatine. Hence, several studies have shown that urinary creatinine is correlated with urinary arsenic in various populations, which makes the interpretations of creatinine adjusted arsenic difficult.

As the reviewer has rightly commented, creatinine concentrations seem to be associated with arsenic metabolism, along with other variables, such as sex, muscle mass and ethnicity. In order to try to control the present analysis we have used the approach proposed by Barr et. al, which involves including the creatinine concentrations in the multivariate models of factors associated with arsenic concentrations as a separate independent variable (Barr et al., 2005).

We have added information about creatinine adjusted arsenic as a limitation of the study:

Discussion section (page 22): "*This study has several limitations (…) 4) finally, we used creatinine concentrations to control for urinary dilution. Creatinine concentrations seem to be associated with As metabolism. To control for this effect, we used the approach proposed by Barr et. Al (2005), including the creatinine concentrations in the multivariate models as a separate independent variable. Nevertheless, due to the complexity of the interrelations between As metabolism, micronutrients and creatinine, the interpretation of the factors associated with As metabolism should be taken with caution*".

General comment: Please review the paper carefully in order to correct grammatical errors.

In order to improve the readability and quality of the paper, the manuscript has been revised by a native English speaker.

Reviewer #3:

This is a descriptive study of total arsenic and arsenic species levels among pregnant women in Spain, as well as the environmental, socio-demographic, or dietary factors associated with the different arsenic metabolites. The study also evaluates the arsenic methylation capacity. For the analysis, the authors chose to use a different set of covariates to adjust the regression models based on a two-step approach for selecting the most appropriate covariates. Although I agree that this might improve the power for analysis, I find that it makes a bit more complicated to follow the results presented on the tables and figures. This is just a personal opinion, and not something that the authors should necessarily change.

In addition, I also have several comments.

We are very grateful to the reviewer for his/her careful reading of our manuscript. The authors consider that her/his suggestions have improved the manuscript. The specific points raised by the reviewer are addressed below. We have tracked changes in the text.

*** The authors say in the Introduction that "Other health effects described in adults have been an increased risk of respiratory, cardiovascular and metabolic diseases (Agency for Toxic Substances and Disease Registry, 2016)." Please cite research articles or systematic reviews in addition of the report by the ATSDR.**

As the reviewer suggested, we have added the following references to support the information about exposure to As and health effects in adults.

- Moon K, Guallar E, Navas-Acien A. 2012. Arsenic Exposure and Cardiovascular Disease: An Updated Systematic Review. Curr Atheroscler reports 14:542–555; doi:doi:10.1007/s11883-012-0280-x.
- Sanchez TR, Perzanowski M, Graziano JH. 2016. Inorganic arsenic and respiratory health, from early life exposure to sex-specific effects: a systematic review. Environ Res 147:537–555; doi:10.1016/j.envres.2016.02.009.

*** I believe "fetus" and "fetal" (instead of "foetus" and "foetal") is the most accepted form in the scientific community.**

As recommended by the reviewer, we have modified these terms in the manuscript.

*In the Introduction, the sentence "during pregnancy in Spain" seems too ambitious to me. I think something like "in a cohort of Spanish pregnant women" (or similar) would sound better.

We thank to the reviewer the suggestion. We have reworded this sentence in the objectives of the study.

Introduction section (page 6): "*The aim of this study is to describe the concentrations of total As (TAs) and the different urinary As species (DMA, MMA, AB and iAs) and the methylation capacity ~~during pregnancy~~ ~~in Spain~~ in a birth cohort of pregnant Spanish women*".

* Supplemental Material table 1. Regarding superscript "c", authors say data represent median and interquartile range. Is this correct? For median (IQR) it is commonly used the format "median (q1, q3)".

Following the reviewer´s suggestion, we have presented the median and p25-p75 in Table S1.

* Authors calibrated inorganic arsenic and arsenic species by arsenobetaine in order to better account for inorganic arsenic exposure. For each arsenic metabolite, they did so by using a residual based method, in which they added the "mean level of each metabolite estimated from participants with AB<1µg/L". I am curious, how many women had AB levels <1ug/L? Also, why did not the authors calibrate total arsenic as well?

We used this approach based on the study by Jones, 2016 (PMID: 27702745). The authors of this study calculated the calibrated concentrations of each As metabolite using the cut-off point of AB of 1µg/L. In our analysis we tested other cut-off points, because to the percentage of participants in our sample with AB levels below 1µg/L was low (n=42; 4.13% of the total sample). Nevertheless, we made the decision to follow the method proposed by the authors exactly, in order to increase the comparability of the data.

In our study we have used this method to calibrate the As metabolites concentrations (MMA, DMA and iAs). In this way, we wanted to try to eliminate the influence of DMA from arsenolipids and arsenosugars in order to carry out a more accurate assessment of both exposure to iAs (sum of MMA, DMA and iAS concentrations), as well as its metabolism.

However, we also wanted to have a global view of exposure to any form of As (organic and inorganic), so we did not calibrate Total As.

*The Statistical Analysis section should be reorganized to better explain the order of analysis performed and the tables and figures. For instance, the recalibration of arsenic metabolites was done before the descriptive and the regression models.

As the reviewer has suggested, we have reorganized the statistical section. We have placed the calibration description before the descriptive and the regression models in order to improve the readability of the manuscript.

* Table 1. Please unify the format to separate the numbers across the table.

We thank the reviewer for noticing the error in the format. We have made the changes to unify the format in order to separate the numbers.

* Table 1. For metabolite percentages it does make sense to have both options, ug/L and ug/g, since the these are percentages and not concentrations.

We have removed the As metabolites percentages calculated with As concentrations adjusted by creatinine.

*Re-wording of some sentences might be helpful. For instance, the sentence "The multivariate models for the factors associated with the concentrations for each of the measured As species can be observed in Figure 1", is not fully correct, since what you are actually showing are the results of those models, but not the models themselves.

We have added the reviewer's suggestion in the tittle of Table 3 and Figure 1: "*Table 3: Beta coefficient (95%CI) of the multivariate linear regression …*"

* Figure 1. Please explain the meaning of the points and the segments. I know they are beta estimates and 95% CI, but it should be specified somewhere in the figure.

We have added this information "Beta coefficient (95%IC)" to the graphic.

* Unify the use of µg/g, µg/gr or µg/gram across the manuscript.

We have checked to ensure the same nomenclature is used throughout the manuscript.

ARTÍCULO III

Manuscript Number: ER-21-2898

MS Title: Prenatal As exposure and methylation efficiency and neuropsychological development among pre-school children in a Spanish birth cohort.

Revised tittle: Prenatal arsenic ~~As~~ exposure, ~~and~~ arsenic methylation efficiency, and neuropsychological development among pre-school children in a Spanish birth cohort

Reviewer #1

Comments for authors

In the present work, the authors evaluated relationship between prenatal total As concentrations (TAs), the As species and the methylation efficiency, and child neuropsychological development in a INMA, Spanish birth cohort.

This study definitely gives very important, extensive and new data on prenatal As species concentrations and methylation efficiency and its association with child neurodevelopment (adjusting other metals and essential elements) from a considerably large sample.

However, I am not very sure if authors could utilize available epidemiological knowledges (specially dose response and time lag of association) and justify/interpret their results well. In addition, I have few concerns to be address by authors before any recommendations.

We are very grateful for the reviewer´s careful reading of our manuscript, which has allowed us to clarify some aspects of it. The specific points raised by the reviewer are addressed below. We have tracked changes in the text.

Section specific Comments/Suggestions

Introduction

1. Authors did not debate dose response in reported association together with time-lag effect. Literature review in effect of prenatal arsenic (As) in neurodevelopmental indicator in different exposure level and duration of follow up would be very helpful for readers.

As the reviewer has suggested, we have added a paragraph in the Introduction section about the time-lag effects of prenatal arsenic exposure.

Introduction section, page 6, 2nd paragraph: Moreover, the developing brain seems to be vulnerable to this toxicant (Grandjean and Landrigan 2006). The development of the central nervous system (CNS) is a process that begins very early in embryogenesis and continues until adolescence (Stiles and Jernigan 2010). As and its compounds, which are able to cross the blood brain barrier, could produce neurotransmitter impairment, brain cell death and degeneration of CNS, among other toxic effects (Piao et al. 2015; Tolins et al. 2014). These prenatally produced changes seem to increase the susceptibility to develop negative effects in the neuropsychological development throughout childhood (Grandjean and Landrigan 2006; Piao et al. 2015).

We also added the exposure levels of the different studies found in the literature review. The follow-up of these studies (time of pregnancy of As assessment and evaluation of the children's age of neuropsychological development) are in the introduction (page 6, 2nd paragraph).

Method

1. Since the age of 5 is very active phase of life and kids can be assumed very shy? How about MSCA assessment environment? where the participants were evaluated? Did they maintain uniform assessment condition (i.e., presence of siblings, members of family, outsides, white coat)? Six testers were involved; So, can we know about inter-tester variability and test-retest variability?

The children were evaluated in the reference hospital. The application conditions followed the indications recommended in the Spanish adaptation of the manual of the MSCA (reference below). Briefly, the protocol included the following indications:

- The scale was administered in a quiet room, with a table cleared of material, with the psychologist positioning him/herself appropriately in front of the child. He/ she did not wear a white coat or similar.

- The presence of the mother or other relatives was not allowed.

- The child should be calm and rested. If the child was ill, not rested, or very negative / oppositional to the test, an attempt should be made to reschedule it for another day. Children who presented special conditions (behavioural problems, sleepiness or being feverish) were classified in a different category and were excluded from the multivariate models as sensitivity analysis. The results obtained were virtually the same and can be consulted in the Supplementary material (Figures S2-S4).

Reference:

Dorothea McCarthy; Agustín Cordero Pando; et al. 2006. MSCA: escalas McCarthy de aptitudes y psicomotricidad para niños : manual. TEA Ediciones: Madrid.

Regarding inter-tester variability, a strict protocol was applied to avoid inter-observer variability. This protocol included inter-observer training and three sets of quality control (inter-observer reliability tests) during the field work. The inter-observer variability assessed by Pearson correlations was lower than 5%.

We have added this information in the manuscript:

Material and methods section, page 9, 2nd paragraph, 2.2.2 Outcome variable: neuropsychological assessment: Testing was conducted in the research centres of each area of study by six psychologists using a strict protocol to avoid inter-observer variability. This protocol included inter-observer training and sets of quality control (inter-observer reliability-tests). The inter-observer variability assessed by Pearson correlations was lower than 5%. The raw scores were standardized for the child's age at test administration and for psychologist. In order to homogenize scales, standardized residuals were then typified by having a mean of 100 and a standard deviation of 15 points.

2. Which food composition table was used to estimate energy adjusted dietary intake of arsenic as well as folate, vitamins B12 and B6 intake?

For the adjusted dietary intake of vitamins B6 and B12 and folate the food composition tables of the US Department of Agriculture and other Spanish sources were used (see the references below).

References:

- *U.S. Department of Agriculture: Agricultural Research Service USDA. National Nutrient Database for Standard Reference, Release 25. [Internet]. 2007. Available from: https://www.ars.usda.gov/ARSUserFiles/80400525/Data/SR25/sr25_doc.pdf*
- *Palma I, Farran P, Cervera P. Tables of Food Composition for household measures of habitual consumption in Spain: CESNID. 4th ed. Madrid: McGraw Hill; 2008.*

We have added this information in the manuscript.

Material and methods section, page 10, 1st paragraph, 2.2.3 Covariates and potential confounders: Additionally, dietary folate and vitamins B_{12} and B_6 intake were also estimated using the food composition tables of the US Department of Agriculture (U.S. Department of Agriculture: Agricultural Research Service USDA 2007) and with Spanish sources (Palma et al. 2008). The intake of supplements was added to the calculation of total daily nutrient intake (Vioque et al. 2013).

How did they adjust for different portion sizes and frequencies by participant kids without weighted food record (WFR) and 24 hour recall (24-h recall)? Estimate may not be precise without WFR and 24 h recall?

In this study, we used maternal dietary variables collected at the same time as the urine samples, but not the kid's dietary information. The women´s dietary intake was assessed in the first trimester of pregnancy using a semi-quantitative food frequency questionnaire (FFQ). The FFQ was previously validated in part of

the study population (740 Valencian pregnant women). For the validation of the FFQ, the reproducibility and validity against biochemical biomarkers were evaluated (more information about this method is available in the reference below).

Reference:

Vioque J, Navarrete-Muñoz E-M, Gimenez-Monzó D, García-de-la-Hera M, Granado F, Young IS, et al. 2013. Reproducibility and validity of a food frequency questionnaire among pregnant women in a Mediterranean area. Nutr J 12:26; doi:10.1186/1475-2891-12-26).

3. HOME scale is one of the well suggested factors to be included to know postnatal growth environment (not only learning materials, toys, but lo care givers attitude, response to kids) very critical for cognitive development in this age? In this study, why this is not included? Is it a limitation of study, or author considered some other proxy variables instead?

Unfortunately, we do not have the HOME scale in the both INMA cohorts. We have tried to adjust the models with well-known variables related to cognitive development, i.e., parental education level, maternal working status, main caregiver at 5 years of age, attendance at nursery school, maternal verbal intelligence, parental social class, etc.

Therefore, we have added this fact as a limitation of our study.

Discussion section, page 21, 2nd paragraph: Finally, the present study lacks information on some important variables that could have an influence on cognitive development (such as the postnatal growth environment), and on the relationship between As methylation and child neurodevelopment, such as other nutrients involved in one-carbon metabolism ~~OCM~~ (i.e. choline and betaine) and genetic information.

Results

1.I like "table 1" to present characteristics of participants in context of other related earlier cohort.

We have added a new Table 1, with the descriptive analysis of some of the sociodemographic and environmental characteristics of the study population. In this table, we have also included the maternal urinary TAs and ∑As concentrations by the different characteristics, in accordance with a suggestion made by another reviewer. Additionally, the descriptive analysis of the information collected at 1 year and 4–5 years of age can be found in Table S1.

2. Fig 1-3 looks busy, can author differentiate significant Beta with not significant by Bold beta value or different color (i.e., red for inverse or green for positive).

In order to improve the interpretation of the figures, we have changed the colours of the coefficients according to the p-value.

3. Despite large sample size, can author consider Bonferroni correction as lots of tests (specially in interactions Figure 1-3) were done (for the scenario of chance finding)? Further, only associations significant at p<0.01 can be an option to consider significant and discuss instead of variables significant at p<0.05 level.

We understand the reviewer comment regarding multiple comparisons, particularly in the interaction analysis. However, many methodological experts feel that Bonferroni or other corrections are not generally warranted (Rohtman 1990). Rather than emphasize statistical significance, they suggest focusing on the magnitude and direction of associations observed, as well as the width of the confidence intervals. Following the recommendations of general guides for reporting research studies (Vandenbroucke, 2007; Moher, 2010), we have left the main analysis unchanged, and all results have been provided in the results or supplemental sections (not only subsets selected a posteriori). Readers concerned with a particular relationship can readily evaluate the extent to which each coefficient meets their criteria. From this point of view, no 'multiple comparisons' were performed since each of them stand alone as they refer to different compounds with particular characteristics, and different outcome variables describing specific neurodevelopmental dimensions. Additionally, corrected p-values would also lead to an artificial increase of the likelihood of Type II error and a reduced comparability with other studies, since the corrected p-value for each association varies with the number of tests performed with other unrelated variables

References:

-*Rohtman KJ. No adjustments are needed for multiple compatisons. Epidemiology. 1990;1:43-6.*

-*Vandenbroucke, J.P., Von Elm, E., Altman, D.G., Gøtzsche, P.C., Mulrow, C.D., Pocock, S.J., et al. 2007. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE): explanation and elaboration. Annals of Internal Medicine, 147(8), W-163.*

-*Moher, D., Hopewell, S., Schulz, K.F., Montori, V., Gotzsche, P.C., Devereaux, P.J., et al. 2010. CONSORT 2010 Explanation and Elaboration: Updated guidelines for reporting parallel group randomised trials. J Clin Epidemiol 63:e1-37.*

That said, we agree with the reviewer in the general risks associated with multiplicity of analysis and it has been recognized in the discussion section of the manuscript:

Discussion section, page 21, 2nd paragraph: In the present work, multiple analyses were performed, particularly in the interaction analysis, therefore results should be taken with caution because some significant associations could result from chance. Coefficients and their confidence intervals should be taken as a global representation of the pattern of the relations between the variables involved in the study (Rothman 1990).

4. Authors may consider making one table (not figure) with significant results only at p<0.01 level for the ease of reader. If possible, I request to discuss such results only considering large number of tests without any adjustment (e.g. Bonferroni correction) for chance finding.

As we have commented above, we have tried to improve the readability of the figures by changing the colours of the coefficients according to the p-value. Furthermore, we have also added three tables in the supplementary material with the beta coefficients and 95%CI of all the interactions, highlighting with a colour those results with a p-value <0.05 and with another colour those with a p-value <0.01.

We have also discussed only the associations with a p-value <0.01 in the interaction analysis and we have added as a limitation the risk associated with multiplicity of analysis.

Discussion

Well written discussion. Enjoyed reading it.

1. However, while comparing TAs association with earlier studies, it will be better to compare dose response (for TAs at least) too specially comparing studies to locate/ judge the participant's exposure level.

So, that we can compare and interpret finding safely.

We thank the reviewer for his/her suggestion. We have now added the median As concentrations of the different studies to the text. We have also added the following sentence:

Discussion section, page 16, 1st paragraph: Arsenic concentrations were substantially different across the studies; for example, the median urinary ∑As concentrations in the Bangladeshi cohort of pregnant women were around 80 µg/L, which are much higher concentrations than in the present study (7.4 µg/L). These differences in urinary ∑As concentrations could imply a differential relative contribution of each species in the total concentrations.

2. Can authors check if positive associations still remain between TAs and AsB among participant who did not consume fish? Since FFQ might have failed [as assumed by myself (Methods#2) earlier and authors) to catch such variation.

We have answered this suggestion in the following comment.

3. Positive association between neurodevelopmental indicators scores and iAs, TAs and AsB need further investigation with separate analysis as suggested in earlier #2 points if possible.

We thank the reviewer for his/her suggestion. In the current analysis, both multivariate models were adjusted by seafood consumption. Moreover, we have performed an additional analysis. We have run the models of TAs and AB as exposure variables and the verbal scale as an outcome, in the sub-sample of

pregnant women with the least seafood consumption (first quartile of seafood consumption= 0 to 25.22 grams per week, n= 201). We have chosen this subsample because there were very few women who did not consume fish (n=22).

The results are the following:

Outcome	Exposure	Model	n	Beta (95%CI)	p-value
Verbal scale	log2 TAs	1	807	0.65 (0.03, 1.27)	0.04
	log2 TAs	2	201	1.01 (-0.16, 2.19)	0.09
	log2 AB	1	807	0.59 (0.11, 1.07)	0.02
	log2 AB	2	201	0.78 (-0.06, 1.62)	0.07

Model 1 (analysis presented in the manuscript): total sample, adjusted by maternal age. maternal body mass index (BMI) before pregnancy. maternal place of birth. maternal and paternal educational level. maternal working status during pregnancy. type of area of residence. Parity, child's sex, season of sample collection, and seafood consumption in first trimester of pregnancy.

Model 2 (additional analysis): subsample of women in the 1st quartile of seafood consumption, adjusted for the covariables of Model 1, except for seafood consumption.

The positive coefficient and the p-value of the association in model 1 (total sample) could be indicating that TAs and AB concentrations possibly acted as a proxy of part of the fish intake variability that is beneficial for brain development. In fact, better performance in the MSCA test associated with higher maternal fish consumption during the first trimester of pregnancy has already been reported in our population (Julvez, 2016). The significance of these positive coefficients was attenuated when we ran the same models using the subsample of women with lower fish consumption (model 2). There could be two possible explanations for this: 1) this measurement error of the maternal fish intake variable obtained by FFQ is lower among this sub-group, or 2) the reduction in the sample size. The fact that even adjusting for maternal fish consumption, a direct marginal association between TAs and AB concentrations and the verbal scale is maintained (model 1) might indicate some degree of misclassification of the maternal fish intake variable not fully captured by the questionnaire. The FFQ is a good instrument method for assessing and classifying mothers according to their usual dietary intake. However, it might suffer from measurement error due to the general difficulties in evaluating regular dietary intake: memory bias, portion sizes, etc. Measurement error in confounders has been shown to affect effect estimates of exposure variables.

We have added this analysis as a sensitive analysis in the Results section and the Supplementary material.

Results section, page 13, last paragraph: Finally, in order to investigate the positive association between maternal urinary TAs and AB and the scores of the verbal scale, we ran the main models in a sub-sample of pregnant women with the least seafood consumption (first quartile of seafood consumption = 0 to 25.22

grams per week, n=201). The positive coefficients were maintained but the p-value of the associations did not reach statistical significance (see Table S5).

This point was previously described discussed in the manuscript.

Discussion section, page 19, 1st paragraph: We have also observed a direct association between both TAs and AB concentrations and scores for the verbal scale. Fish and seafood consumption is the main source for the organic form AB (European Food Safety Authority 2009), and is the highest contributor to the total As concentrations. Even though fish consumption is a source of toxicants (i.e. As, but also methylmercury and polychlorinated biphenyls), it also provides some essential nutrients related to better neurodevelopment, such as proteins, vitamin D and, especially, n-3 long-chain polyunsaturated fatty acids (LCPUFA) (FAO/WHO 2010; Julvez et al. 2016). Although the models were adjusted for fish consumption, it is possible that the food frequency questionnaire did not fully record the maternal fish intake variability, thereby introducing some degree of misclassification.

Reference:

-*Julvez J, Méndez M, Fernandez-Barres S, Romaguera D, Vioque J, Llop S, et al. 2016. Maternal consumption of seafood in pregnancy and child neuropsychological development: A longitudinal study based on a population with high consumption levels. Am J Epidemiol 183:169–182; doi:10.1093/aje/kwv195.*

4. As discussed about the role of Mn in As toxicity, authors may again compare the dose of INMA study, and that of two epidemiological studies [i.e., US study by Wright et al. (2006) and that of Bangladesh study by Valeri et al. (2016)]. So that, if Mn level in Spain, USA and Bangladesh differ for different effect.

Following the reviewer's previous recommendation, we have discussed only the associations with a p-value <0.01 in the interaction analysis. Due to this change, we have eliminated the paragraph of the Mn interaction.

5. So, what is the interpretation/assumption of authors regarding lower scores on the general, verbal and memory scales among Children whose mothers presented increasing %iAs and iron deficiency (ID) (ferritin <15 mg/dL)? Are authors assuming Iron deficiency causing low methylation or they are not sure? Authors already (Page 15, Line 60) suggested high %iAs means lower proportion of %MMA or %DMA?

In our study, we observed that children whose mothers presented increasing %iAs and ferritin levels <15 µg/L obtained lower scores on several MSCA scales.

When we reviewed the literature about this topic, we found only three epidemiological studies: two cross-sectional studies (conducted in children from Uruguay and Mexico) and one Bangladeshi cohort of pregnant women. However, they only assessed the effect of Fe on As metabolism; none of these studies evaluated the influence of the interaction between the two metals on children's neurodevelopment. The results obtained by these three studies were heterogeneous. While in the Mexican study higher serum

ferritin was related to higher As methylation efficiency (denoted by lower %MMA and higher %DMA), in the Uruguayan study the association was the opposite (iron deficiency was related to lower %iAs and higher %DMA). Finally, no significant association was observed in the cohort of pregnant women from Bangladesh. Due to the scarce evidence currently available, the authors cannot be sure whether or not iron deficiency could improve the methylation capability or how these two elements could interact with each other. Our results suggest a possible influence of iron status on As metabolism and toxicity, although the authors believe that this relationship should continue to be investigated.

6. Though this study aimed to see effect of prenatal As exposure on 5 years kids, we may assume lots of postnatal exposure at the age of 5 which might be acting together /masking the effect. In addition, early damage of brain development may persist long but "neuroplasticity of brain" might have acted up on. Further, as authors already listed as limitation, the single urine samples at 1st trimester may not represent the prenatal exposure. Finally, though education of mother and attendance at nursery included in core model, postnatal growth environment (e.g., HOME Environment) was not considered, which is mostly adjusted in many similar studies.

We thank the reviewer for his/her considerations. The authors agree with the reviewer that the lack of information on postnatal As exposure and postnatal growth environment suppose a limitation in our study. We have added this fact as a limitation of our study.

Discussion section, page 21, 2nd paragraph: Another limitation is the assessment of As exposure at only one time-point during pregnancy. As the vulnerability of the central nervous system extends from the beginning of pregnancy to adolescence, and As metabolism efficiency seems to change during pregnancy, it would have been more accurate to measure As and its metabolites at several time-points during pregnancy, as well as in postnatal period.

Authors may remove one extra page at the end of Supplementary File.

We have removed the extra page at the end of the Supplementary File.

Despite few concerns noted above, very extensive/comprehensive work has been done and the manuscript is well written. I hope, the authors will address these concerns to give its message more effectively.

The authors thank the reviewer for all his/her considerations and suggestions.

Reviewer #2

The manuscript by Soler-Blasco et. al. focuses on the association between maternal urinary arsenic concentrations and arsenic methylation capacity assessed in the first trimester of pregnancy and children's cognitive outcomes at the age of 5 years. This prospective cohort study has several strengths and adds to the scarce knowledge in this topic area. Addressing the following comments will help strengthen the manuscript and clarify certain aspects further.

We thank the reviewer for all his/her suggestions and the recommended references. The authors have tried to improve the manuscript by following the reviewer's suggestions. The specific points raised by the reviewer are addressed below and we have tracked changes in the text.

General comments applicable to the whole manuscript:

The words "negative relation" are used in describing the results obtained from previous studies. Please replace "negative relation" with "inverse association".

As the reviewer has suggested, we have changed the form "negative relation" to "inverse association" along the manuscript

Please review the manuscript thoroughly for grammar and focus on the use of appropriate prepositions. For example, the last paragraph of the introduction (line 27) - "methylation efficiency at first trimester" - "at" should be replaced by "in". Similarly, in paragraph 8 of the discussion section, line 26/27 includes "...relationship between arsenic and iron on the human body..."; please replace the preposition "on" with "in". Please check for similar corrections elsewhere.

We thank the reviewer for noticing these mistakes. In order to improve the readability and quality of the paper, the manuscript has been revised by a native English speaker.

Highlights:

Highlights should include that As assessments were done in urine samples, example, "prenatal MMA" can be reworded as "prenatal urinary MMA".

As the reviewer has suggested, we have added the word "urinary" in the first highlight.

Last highlight - children obtained lower scores on cognitive tests; just stating "lower scores" is not clear

As the reviewer has suggested, we have added "lower scores on cognitive tests" in the last highlight, in order to clarify the sentence.

Title:

The title should not contain an abbreviation (As). Please write the full form.

We have changed the abbreviation As to the full form in the title.

Consider rewording the title as follows "Prenatal arsenic exposure, arsenic methylation efficiency, and neuropsychological development among pre-school children in a Spanish birth cohort".

We have reworded the title as the reviewer has suggested.

Abstract:

Objectives - Clarify that the total As concentrations are urinary As concentrations.

We have clarified that the total As concentrations were measured in urine.

Materials and Methods - Write the full form of INMA at its first use.

We have added the English full form of the INMA abbreviature (Childhood and Environment).

Line 21: "Neuropsychological development was assessed at 4-5 years-old" needs to be changed to "Children's neuropsychological development was assessed at the age of 4-5 years".

As the reviewer has suggested, we have reworded this sentence in the abstract.

Line 28: Maternal nutrients and maternal vitamin intake - clarify that the nutrients were measured in terms of nutrient status in serum, not as intake.

As the reviewer has suggested, we have added the following information in the abstract.

Abstract, Materials and Methods section: We explored effect modification by sex, iron status, maternal nutrients status (serum manganese and selenium, and urinary zinc), and maternal vitamins intake (folate, and vitamins B_{12} and B_6).

Introduction:

Line 29: Add commas between Cd, Se, Zn

We thank the reviewer for noticing this mistake. We have added the commas.

Paragraph 4: Please add the mean/median exposure levels in the previous studies that are described; that will help in gaining a perspective of the study findings in different areas of the world as the reader compares the study findings.

As the reviewer has suggested we have added this information in the Introduction section. We have also added the As concentrations of the different studies cited in the Discussion section

Methods:

Study population - The fact that participating mothers were older than non-participating mothers is a result, not a method. Please move that sentence to the results section.

As the reviewer has suggested, we have moved this sentence to the Result section.

Results section, page 13, 1st paragraph, 3.1 Description of TAs and its metabolite concentrations and As methylation efficiency: Differences between included and excluded subjects are shown in Table S1. The participating mothers were slightly older and had a higher level of education and social class than the non-participants.

Please add a subsection that details the urinary creatinine assessment technique and clarify that the urinary arsenic concentrations were adjusted for urinary creatinine.

We have added some information about the urinary creatinine assessment technique:

Material and methods section, page 9, 2nd paragraph, 2.2.3 Covariates and potential confounders: Serum manganese (Mn) and selenium (Se), urinary cadmium (Cd) and zinc (Zn), and plasma ~~plasmatic~~ ferritin concentrations were determined from the first trimester of pregnancy. More information about the methodology has been reported in detail elsewhere (Arija et al. 2019; Lozano et al. 2020; Soler-Blasco et al. 2020). Creatinine concentrations were measured in the same urine samples in the first trimester of pregnancy by the DRI® Creatinine-Detected® Test using AV680 from Beckman Coulter.

In the statistical analysis section, the adjustment of the As concentrations by urinary creatinine was previously described:

Page 9: Arsenic methylation efficiency was determined through two approaches. The first consists in calculating the percentage of the individual calibrated iAs, MMA and DMA over the sum of those species (∑As). The second involves a principal component analysis (PCA) of the three calibrated, untransformed and un-rotated percentages corrected for maternal creatinine.

Page 10: We calculated the geometric mean (GM) and 95% confidence intervals (95%CI) of the urinary creatinine adjusted (µg/ g creatinine) TAs, AB, DMA, MMA, iAs and ∑As concentrations (as the sum of iAs, DMA and MMA).

Page 10: The area of study (Valencia or Gipuzkoa) and maternal urinary creatinine concentrations were included in all core models regardless of their statistical significance.

Neuropsychological assessment - Was this conducted in the school/home of the participant/research center? Please clarify.

The neuropsychological assessment was conducted in the research centres in each area of study (in both cases in a quiet room, with a table cleared of material, with the psychologist positioning him/herself appropriately in front of the child).

We have added this information in the manuscript

Material and methods section, page 8, 2nd paragraph 2.2.2 Outcome variable: neuropsychological assessment: Testing was conducted in the research centres of each area of study by six psychologists using a strict protocol to avoid inter-observer variability.

Covariates and potential confounders - Lines 43-44 state that questionnaires were filled by the women during pregnancy and then after delivery up to 4-5 years. Please clarify whether the questionnaires were filled each year up to 4-5 years post delivery, or just once after 4-5 years post delivery.

We have added information about when the questionnaires were filled in.

Material and methods section, page 9, 1st paragraph, 2.2.3 Covariates and potential confounders: Socio-demographic, environmental and lifestyle information was collected through questionnaires filled during pregnancy and, ~~and~~ later at 12-14 months of age and at ~~after delivery up to~~ 4-5 years.

Dietary assessment - Lines 54-55 describe how the FFQ was used. It is stated that the grams/day intake of foods such as rice, meat, seafood etc. was estimated by the researchers. Were questions about these foods not part of the FFQ? If they were, please reword the sentence and avoid using "we estimated".

We used an FFQ that had nine possible responses for each item, ranging from 'never or less than once per month' to 'six or more per day' (Vioque, 2013). A commonly used serving size was specified for each food item in the FFQ (including rice, meat, seafood, etc.). This was converted to average daily intake in grams for each individual participant. We have changed the word "estimated" to "calculated" in order of avoid misunderstandings.

References:

Vioque J, Navarrete-Muñoz E-M, Gimenez-Monzó D, García-de-la-Hera M, Granado F, Young IS, et al. 2013. Reproducibility and validity of a food frequency questionnaire among pregnant women in a Mediterranean area. Nutr J 12:26; doi:10.1186/1475-2891-12-26.

Authors also state that the dietary intake of B-vitamins was estimated. Was this done using a dietary database? If so, please provide details of which database was used and how the intakes were calculated.

Dietary intake of vitamins B_6 and B_{12} and folate was calculated using the food composition tables of the US Department of Agriculture and other Spanish sources (see references below).

References:

U.S. Department of Agriculture: Agricultural Research Service USDA. National Nutrient Database for Standard Reference, Release 25. [Internet]. 2007. Available from: https://www.ars.usda.gov/ARSUserFiles/80400525/Data/SR25/sr25_doc.pdf

Palma I, Farran P, Cervera P. Tables of Food Composition for household measures of habitual consumption in Spain: CESNID. 4th ed. Madrid: McGraw Hill; 2008.

We have added this information in the manuscript.

Material and methods section, page 9, 1st paragraph, 2.2.3 Covariates and potential confounders: Additionally, dietary folate and vitamins B_{12} and B_6 intakes were also estimated using the food composition tables of the US Department of Agriculture (U.S. Department of Agriculture: Agricultural Research Service USDA 2007) and with Spanish sources (Palma et al. 2008). The intake of supplements was added to the calculation of total daily nutrient intake (Vioque et al. 2013).

Statistical analysis - Please add a few sentences describing descriptive analyses at the beginning of the section.

In the third paragraph of the Statistical analysis section, we described the descriptive analysis of the urinary creatinine adjusted Total As and As species. We have moved this paragraph to the beginning of the section.

Multivariable linear regression models - was the variable for maternal urinary creatinine concentrations added to the models in addition to the arsenic concentrations being already creatinine-adjusted? What was the rationale behind doing this? This method raises concerns of over-adjustment.

In the multivariable regression models, when the exposure variables were the urinary As concentrations, the variables used were not adjusted for creatinine. The urinary creatinine concentrations were included in multivariate models as a separate independent variable.

In order to avoid misunderstandings, we have added this information in the manuscript:

Material and methods section, page 11, last paragraph, Statistical analysis: In the second step, each As variable (uncalibrated, unadjusted for urinary creatinine and log2 transformed TAs, ∑As, AB, DMA, MMA and iAs concentrations, calibrated and probit-transformed DMA, MMA and iAs percentages, and PC1 and PC2) were included in these models as exposure variables.

Further, N-guanidinoacetate methyltransferase, which is the main consumer of methyl groups from the human one carbon cycle, participates in the formation of creatinine from creatine. Hence, urinary creatinine is correlated with urinary concentrations of arsenic metabolites. So, specific gravity adjustments are preferred over creatinine adjustments for urinary arsenic. Some previous papers from the INMA cohort have used specific gravity. Why was that not used in this analysis?

As the reviewer has commented, there is a publication from the INMA cohort in which specific gravity was measured and it was used for the adjustment of urinary arsenic (Signes-Pastor, 2017). Nevertheless, this measurement was only analysed in a subsample of 100 pregnant women. Unfortunately, we did not have specific gravity measurements for the whole sample.

The authors are aware of the limitation of adjusting the urinary arsenic concentrations for urinary creatinine because, as the reviewer has rightly commented, creatinine concentrations seem to be associated with arsenic metabolism, along with other variables, such as sex, muscle mass and ethnicity. In order to try to control the present analysis we have used the approach proposed by Barr et. al (see reference below), which involves including the creatinine concentrations in the multivariate models as a separate independent variable.

Reference:

Barr DB, Wilder LC, Caudill SP, Gonzalez AJ, Needham LL, Pirkle JL. Urinary creatinine concentrations in the U.S. population: implications for urinary biologic monitoring measurements. Environ Health Perspect. 2005 Feb;113(2):192–200.

Signes-Pastor AJ, Carey M, Vioque J, Navarrete-Muñoz EM, Rodríguez-Dehli C, Tardón A, et al. Urinary arsenic speciation in children and pregnant women from Spain. Expo Heal. 2017;9(2):105–11.

Line 23-28 in paragraph 4 detail the second step of the multivariable regression model building - were all of those As metabolites and other variables added simultaneously to the model? If so, why were the transformed and untransformed variables added together?

As regards the construction of the multivariable regression models, first we built a core model with the sociodemographic variables associated with the outcomes (the ten MSCA sub-scales). In the second step we included each exposure variable separately in different multivariate models and then we added the potential confounders for each model.

Statistical Analysis Paragraph 5 - "Effect modification was performed by interacting the exposure variable with the potential modifier" - please reword this sentence to clarify that interaction terms were created between the exposure variables and the potential effect modifiers.

As the reviewer has suggested, we have added this information in the manuscript:

Material and methods section, page 12, Statistical analysis, 2nd paragraph: Effect modification analysis was performed by creating an interaction term between ~~interacting~~ the exposure variable ~~with~~ and the potential modifier: child's sex, maternal nutrients categorized by the median: Mn (< and ≥ 1.44 µg/L), Se (< and ≥ 79.8 µg/L) and Zn (< and ≥ 363.7 µg/L), intake of vitamins B_6 (< and ≥ 2.3 mg/day) and B_{12} (< and ≥ 9.4 mg/day), ferritin (categorized as iron deficiency [ID] as < and ≥ 15 µg/L), and intake of folate (categorized by the intake recommendation during pregnancy, < and ≥ 600 µg/day]. The models with and without interaction term were compared with the Likelihood Ratio test (LRT) and the effect modification was considered statistically significant if the p-value <0.05.

Statistical Analysis Paragraph 5 - Likelihood ratio tests were used to compare the models with and without the interaction terms. Likelihood ratio tests compare the fit of models when predictors are added/removed. What exactly was inferred when the LRTs were observed with/without the interaction terms? Were decisions about which variables to use as effect modifiers based on the LRTs?

This statement means that the interaction term was added to the main models and the likelihoods of the main models and the main models plus the interaction term were compared using the LRT. The 'main effects' (variables which we test for interaction) are already included in the main models, so, we only test for effect modification. This is a standard and recommended method to compare nested models and provides almost identical results than the t-test which is used to construct the confidence intervals of the interaction terms.

Both approaches (chi-square of the LRT and t-test) provided essentially the same results.

The interactions tested were pre-specified and justified in the introduction section and results for all of them are provided in the results section.

Statistical Analysis Paragraph 6 - Lines 57-58 - the authors state that GAMs were used to assess the associations between the MSCA sub-scales and As exposure. Please reorder the exposure and outcome variables in that sentence and state that the associations between As exposure and scores on the MSCA sub-scale were assessed.

We thank the reviewer for noticing this mistake. We have reordered the exposure and outcome variables in this sentence.

Material and methods section, page 12, Statistical analysis, 2nd paragraph: Finally, Generalized Additive Models (GAM) were fitted to evaluate non-linear patterns on the association between As species and scores on the MSCA sub-scales

Results:

Table S1 needs to be brought into the main manuscript tables, not as a supplemental table.

We have added a new Table 1, with the descriptive analysis of some of the sociodemographic and environmental characteristics of the study population. In this table, we have also included the maternal urinary TAs and ∑As concentrations by the different characteristics, in accordance with a suggestion made by another reviewer. Additionally, the descriptive analysis of the information collected at 1 year and 4-5 years of age can be found in Table S1.

We have decided to keep Table S1, which contains the comparison between the included and not included population and the descriptive analysis of the information collected at 1 year and at 4-5 years of age, in the Supplementary Material, because Table 1 would become too large.

Section 3.2 - The results described in lines 20-29 are not statistically significant; the 95% CIs include the null value for beta coefficients (i.e., they include 1.00) Section 3.3, effect modification results - Please change "95% IC" to "95% CI".

In this section we showed only the statistically significant beta coefficients (p-value <0.05). Only one of the coefficients was found to be marginally statistically significant (p-value = 0.06, executive function scale), although we think it should be kept in the text because the association was similar to the others.

We thank the reviewer for noticing this mistake. We have changed "95% IC" to "95% CI".

Discussion:

Paragraph 2, line 35: Please replace "at 10 years old" with "at 10 years of age" and correct for the same in line 55.

As the reviewer has suggested, we have replaced "at 10 years old" with "at 10 years of age" in lines 35 and 55.

Paragraph 2, line 45 - 47: In describing the birth cohort study from China, please clarify that the cord blood arsenic concentrations were used. "As cord serum" is not very clear.

As the reviewer has suggested, we have clarified the matrix where arsenic was measured:

Discussion section, page 16, 2nd paragraph: In the Ma'anshan-Anhui Birth Cohort (China), As cord blood concentrations (serum) (median [p25, p75] = 1.89 [1.27, 2.89] µg/L) were ~~was~~ associated with a higher risk of developmental delay in the social domain among 6-month-old children, assessed through the Ages and Stages Questionnaire.

Paragraphs 2 and 3: Add details of the general As exposure levels in the various studies that are described, how they relate to exposure levels in Spain, and what implications that may have in interpreting the present study findings.

As the reviewer has suggested, we have added the median As concentrations of the different studies to the text. We have also added some implications that the different As exposure levels may have in interpreting the present study findings.

Discussion section, page 17, 1st paragraph: Thus, the epidemiological literature on the relationship between prenatal As exposure and neuropsychological development in childhood is still too scarce to draw any definitive conclusion. Additionally, the methodology used in the different studies is too heterogeneous, which hampers the comparability between them. ~~In addition~~ Furthermore, the biomarker of exposure used in these previous studies was total As (sum of organic and inorganic forms) or ∑As (sum of DMA, MMA and iAs), and none of them evaluated the different species individually. This fact may have added a certain degree of imprecision in the exposure assessment that could partly explain the inconsistencies in the results. Arsenic concentrations were substantially different across the studies; for example, urinary ∑As concentrations in the Bangladeshi cohort of pregnant women were around 80 µg/L, which are much higher concentrations than in the present study (7.4 µg/L). These differences in urinary ∑As concentrations could imply a differential relative contribution of each species in the total concentrations. For example, in the Bangladeshi birth cohort, the major contributor in ∑As may be iAs, due to the fact that the general population in Bangladesh is highly exposed to iAs through drinking water (Shahid et al. 2020). In contrast, in our population, iAs exposure from water is low (around 1 µg/L) (Ministerio de Sanidad Servicios Sociales e Igualdad 2019), and the main contributor to prenatal iAs and its metabolite concentrations is the consumption of certain foods, such as rice or molluscs (Soler-Blasco et al. 2021). Moreover, our population was highly exposed to non-toxic organic arsenicals from high fish consumption.

Paragraph 4: In describing the Mexican study and the Taiwanese study, please add pertinent details, including the sample size, the outcome assessment tools used, and then comment on the comparability of findings with the present study.

As the reviewer has suggested, we have added more information about these two studies. We have also commented on the comparability of findings with the present study.

Discussion section, page 17, 2nd paragraph: Only a few cross-sectional studies have analysed the association between postnatal urinary metabolites and children's cognitive function. In one Mexican study (n=602), the authors found a significant association between urinary MMA concentrations (mean= 7.7 µg/L) and problem-solving, vocabulary, memory and attention tests in schoolchildren, evaluated by several tests (Letter Sequencing, Visual Search, the Peabody Picture Vocabulary Test, the Weschsler Intelligence Scale for Children Revised Mexican Version Digit Span Subscale, among others) ~~(in this study mean MMA concentration was 7.7 µg/L)~~ (Rosado et al. 2007). In a case-control study carried out in Taiwan (n of cases= 63, n of controls= 35), the MMA and the iAs concentrations in the highest tertile (>0.0028, and >0.49 µg/L, respectively) increased the risk of neurodevelopmental delay in preschool children, evaluated through multiple neurodevelopmental tests (Peabody Developmental Motor Scales, Gross Motor Function Measure, Chinese Wechsler Intelligence Scale for Children, and Bayley III Scales of Infant and Toddler Development, among others) (Hsieh et al. 2014). The results derived from these two studies with postnatal As exposure seem to be in agreement than those observed in our study with prenatal As. Nevertheless, comparison of the studies must be performed with caution.

Paragraph 8: Please be consistent in the use of "Fe" and/or "iron".

We have checked to ensure use the same terminology throughout the manuscript.

Paragraph 9: Line 39: The relationship between arsenic methylation efficiency and scores on the MSCA sub-scales was modified by maternal urinary Zn concentrations. Please reword the first sentence to reflect it clearly.

As the reviewer has suggested, we have modified the first sentence of the paragraph:

Discussion Section, page 20, 2nd paragraph: "Finally, we have found that the relationship between As methylation efficiency and scores on the MSCA sub-scales was modified by maternal urinary Zn concentrations ~~we have found an effect modification of the maternal urinary Zn concentrations"~~.

Paragraph 10: The differences in the socioeconomic profile and educational status between those who participated vs. those who did not is indicative of selection bias (in addition to issues of representativeness). Please add a few sentences regarding selection bias in the limitations.

We thank the reviewer for his/her comment. We have added this limitation in the Discussion section.

Discussion Section, page 21, 2nd paragraph: This study has several limitations. Firstly, a considerable proportion of children from the cohort were not included in the present study and this loss to follow-up could represent a selection bias. We observed that the participants who were included had a higher socioeconomic profile and educational level. This fact could be affecting the representativity of the study and the estimation of some exposure- outcome associations.

Paragraph 10: OCM - please use the full form; first time use.

We have changed “OCM” to the full form.

Add a paragraph that includes a comment on the different results obtained for the different sub-scales' scores. For example, executive functions are largely related to the prefrontal cortex of the brain. But the other aspects of cognition might be related to other areas/mechanisms in the brain. Please comment on those and the hypothesized biological mechanisms underlying those associations.

As the reviewer has suggested, we have added a paragraph with some information about mechanism of As toxicity on different areas of the brain, based on different experimental and in vitro studies.

Discussion section, page 18 , 1st paragraph: Although the mechanism of As neurotoxicity is still not fully understood, some experimental studies on animals have proposed oxidative stress as a possible mechanism(Chandravanshi et al. 2018; Luo and Shu 2015); As has been related to an increase in reactive oxygen species (ROS) concentrations and its accumulation in certain areas of the central nervous system, particularly in the frontal cortex region, (Luo and Shu 2015; Mishra and Flora 2008). This accumulation causes lipid peroxidation, which leads to DNA damage and, consequently, brain cell death and degeneration of the CNS (Chandravanshi et al. 2018; Luo and Shu 2015). The prefrontal cortex region is related to memory, perception, new learning, and other cognitive processes (Siddiqui et al. 2008). Another As neurotoxicity mechanism that has been proposed is a neurotransmission impairment, specifically in the metabolism of acetylcholine. The cholinergic alteration produced seems to generate learning and memory impairment (Chandravanshi et al. 2014). The methylated forms, DMA and MMA, seem to be accumulated in the brain. Experimental studies have observed higher levels of MMA in the hippocampus, related to verbal learning and memory, and the thalamus, related to some motor tasks, working memory, attention control, learning and memory processing (Georgescu et al. 2020; Li et al. 2020; Sánchez-Peña et al. 2010; Tyler and Allan 2014).

Conclusions:

Line 50: Please replace "at 4-5 years old" with "at 4-5 years of age".

We have changed this sentence in the Conclusions section.

Reviewer #3

This is a potentially interesting paper to explore the relationship between prenatal total arsenic concentrations, the arsenic species and the methylation efficiency, and child neuropsychological development in a Spanish birth cohort. This is a follow-up study with interesting results, but there are some modifications that need to be made.

We are very grateful to the reviewer for his/her careful reading of our manuscript. The authors consider that changes following her/his suggestions have improved the manuscript. The specific points raised by the reviewer are addressed below and we have tracked changes in the text.

1. Page 8, line 4-33: The score stated in the neuropsychological assessment, authors should explain the meaning of high or low score. In this way, people who are not familiar with this score can also understand.

As the reviewer has suggested, we have added a sentence to explain the meaning of high/low MSCA scores.

Material and methods section, page 9, 1st paragraph, 2.2.2 Outcome variable: neuropsychological assessment sub-section : "The new sub-areas of MSCA used in this study were gross motor and fine motor skills, executive function and working memory (Julvez et al. 2011). Higher scores on the general scale and sub-scales indicate better cognitive development.

2.Page 9, line 48-55: In the PCA analysis, the author should explain clearly how PC1 and PC2 were used as arsenic methylation phenotypes. The author should explain the relationship between PC1 and PC2 and arsenic methylation capacity indices (iAs%, MMA% and DMI%)?

The relationship between the PC1 and PC2 phenotypes is explained in the Results section (subsection 3.1, see below). The authors considered that this information should remain in this section, due to its being a result derived from the analysis.

Results section, page 13, 2nd paragraph, 3.1 Description of TAs and its metabolite concentrations and As methylation efficiency: The variability of the three calibrated percentages of metabolites can be summarized in two principal components. Principal component 1 (PC1) explained 89% of the variance and was characterized by higher %DMA and lower %iAs and %MMA. Principal component 2 (PC2) explained the remaining 11% of the variance and was characterized by higher %iAs and lower %MMA.

Nevertheless, we have added some information explaining the reason for using principal component analysis.

Material and methods section, page 11, 2nd paragraph, Statistical analysis: The second involves a principal component analysis (PCA) of the three calibrated, untransformed and un-rotated percentages corrected for maternal creatinine. The PCA was performed to avoid the high correlation between the percentages of the three metabolites. The results obtained through the PCA were two principal components (PC1 and PC2) that explain 100% of the original variance. Therefore, these two PC were used as As methylation phenotypes.

3. Regarding the study population, if the Materials and Methods section, 2.1 study population included Figure S1 and instructions, readers can clearly know how to recruit study subjects in this study.

As the reviewer has suggested, we have moved Figure S1 (Flow chart describing the process of selecting participants in the INMA Project (Valencia and Gipuzkoa, Spain, 2003-2008) to be included in the present analysis) to the main document as Figure 1.

4. In the Result section, first, there should be a Table presenting the distribution of arsenic species, arsenic methylation indices and various neuropsychological outcome variables based on demographic characteristics of 807 study population. In this way, it is possible to understand which variables can confound the association between arsenic species, arsenic methylation and neuropsychological assessment.

In previous work, we studied the factors associated to the different As species concentrations (Soler-Blasco, 2021). The table requested by the reviewer was already included in the supplemental material of this previous study. In order to avoid overlapping, we have not included this table again, but we have referenced this previous work in the results section

Material and methods section, page 10, 4th paragraph, Statistical analysis: We calculated the geometric mean (GM) and 95% confidence intervals (95%CI) of the urinary creatinine adjusted (µg/ g creatinine) TAs, AB, DMA, MMA, iAs and ∑As concentrations (as the sum of iAs, DMA and MMA). GM and 95%CI of the measured TAs and ∑As were calculated according to sociodemographic, environmental, and dietary characteristics of the study population. Descriptive analyses of the rest of the As species according characteristics of the study population can be seen in Soler-Blasco et.al (2021). For further analysis, we applied the $\log_2$ and probit-transform functions on values of the urinary As species concentrations and the percentage of the individual metabolites, respectively, in order to correct their skewed distribution.

Nevertheless, we have added in the manuscript a new Table 1, with the descriptive analysis of some of the sociodemographic and environmental characteristics of the study population. In this table we have also added the maternal urinary TAs and ∑As concentrations by the different characteristics.

Reference:

Soler-Blasco R, Murcia M, Lozano M, Sarzo B, Esplugues A, Vioque J, et al. Urinary arsenic species and methylation efficiency during pregnancy: concentrations and associated factors in Spanish pregnant women. Environ Res. 2021;196(110889).

5. Figure 3 is too crowded and difficult to understand for readers. Can the author use a clearer diagram or table to show the interaction between maternal nutrients and methylation efficiency on the scores of the ten MSCA scales?

We have tried to improve the interpretability of Figure 3 (now Figure 4), marking the beta coefficients and 95%CI with a p-value of interaction <0.05 (in red colour) and those with a p-value of interaction <0.01 (in brown colour). Furthermore, we have also added three tables in the supplementary material with the beta coefficients and 95%CI of all the interactions, highlighting with a colour those results with a p-value <0.05 and with another colour those with a p-value <0.01.

6. In the statistical analysis section, the correlation of Figure 1 and Figure 2 and the interaction of Figure 3, readers cannot clearly know which confounders were adjusted in each statistical analysis.

The reviewer rightly remarks that the information on confounders and adjustment variables for each model is not found in the figures, making it difficult to read. However, the authors decided to show this information in the supplemental material, due to the fact that it was impossible to include all this information in the figure captions (there are different adjustment variables for each of the models). In the footnote of the figures is indicated where can this information be consulted.

Reviewer #4

The overall objective of the present study was to evaluate relationships between prenatal total As concentrations, the As species and the methylation efficiency, and neurophysiological development in 4-5-year old children (n=807) in the Spanish birth cohort, INMA. In general, the study is clearly described, and the arsenic exposure has been thoroughly assessed, i.e., separating inorganic and organic arsenic. However, there are some major concerns that needs to be addressed.

We thank the reviewer for all his/her suggestions after a careful reading of our manuscript. This has enabled us to clarify some aspects of it. The authors have tried to improve the manuscript by following the reviewers' suggestions. The specific points raised by the reviewer are addressed below. We have tracked changes in the text.

Major comments:

1. The main finding in the present study was that the concentration of MMA was inversely associated with the general cognitive scale as well as with several sub-scales. What does this mean when you consider that i) the exposure to inorganic As (sum As) is very low in the present population and the concentration of MMA is even lower (GM: 0.34 µg/g creatinine), and ii) the authors do not find any association of sum As with any of the outcomes. What would happen if the models of concentration of MMA and outcomes were to be adjusted for the sum of As? In summary, is it really the As exposure which is underlying the associations of MMA and neurophysiological development? Or can MMA reflect something else like an efficient one-carbon metabolism? Earlier studies have found interactions between MMA and tobacco smoking, could this play a role here? This needs to be discussed in more detail. An alternative suggestion would be to only focus on AB and sum of As.

We have answered each question below.

i) As the reviewer has commented, the exposure of inorganic As in our population (sum of DMA, MMA and iAs) is very low, compared with other areas, such as Bangladesh. Nevertheless, at present, there are no established threshold levels below which exposure to arsenic does not produce effects. For this reason, the authors considered that more research is necessary, even in populations with low exposure levels. We consider that the findings of this study are interesting for several reasons: association has only been seen with urinary MMA concentrations, but not with %MMA (only with the memory scale). This result might indicate an incomplete/partial As methylation in our population or an over-exposure to this methylated form through rice consumption. In fact, in our previous work, we found a positive association between

urinary MMA concentrations and rice intake (PMID: 33607098). In addition, detectable levels of MMA in rice have been reported in previous studies (see references below).

References:

-Nookabkaew S, Rangkadilok N, Mahidol C, Promsuk G, Satayavivad J. 2013. Determination of Arsenic Species in Rice from Thailand and Other Asian Countries Using Simple Extraction and HPLC-ICP-MS Analysis. J Agric Food Chem; doi:10.1021/jf4014873.

-Signes-Pastor AJ, Carey M, Meharg AA. 2017. Inorganic arsenic removal in rice bran by percolating cooking water. Food Chem 234:76–80; doi:10.1016/j.foodchem.2017.04.140.

Soler-Blasco R, Murcia M, Lozano M, Sarzo B, Esplugues A, Vioque J, et al. 2021. Urinary arsenic species and methylation efficiency during pregnancy: concentrations and associated factors in Spanish pregnant women. Environ Res 196; doi: https://doi.org/10.1016/j.envres.2021.110889.

-U.S Food and Drug Administration. 2016. Arsenic in Rice and Rice Products Risk Assessment Report.

ii) We have carried out the analysis proposed by the reviewer, including the urinary ∑As concentration (log2-transformed) in the multivariate linear regression model with MMA as the exposure variable. The results can be seen in the table below:

Outcome	Exposure	Model	Beta (95%CI)	p-value
General scale	log2 MMA	1	-1.37 (-2.33, -0.41)	0.01
General scale	log2 MMA	2	-1.47 (-2.54, -0.39)	0.01
Verbal scale	log2 MMA	1	-1.18 (-2.13, -0.23)	0.02
Verbal scale	log2 MMA	2	-1.39 (-2.43, -0.35)	0.01
Quantitative scale	log2 MMA	1	-1.23 (-2.20, -0.27)	0.01
Quantitative scale	log2 MMA	2	-1.34 (-2.40, -0.28)	0.01
Memory scale	log2 MMA	1	-1.19 (-2.17, -0.20)	0.02
Memory scale	log2 MMA	2	-1.60 (-2.70, -0.50)	0.01
Executive function scale	log2 MMA	1	-0.98 (-2.00, 0.04)	0.06
Executive function scale	log2 MMA	2	-1.01 (-2.15, 0.12)	0.08

Model 1: analysis presented in the manuscript, without adjustment by ∑As.

Model 2: additional analysis, with adjustment by log2 ∑As.

As can be observed, the magnitude of the effect and the p-values for the MMA concentrations are similar.

Although no collinearity problems have been observed (VIF<5 in all cases), the variance of some coefficients was increased when including the variable ∑As in the models. In addition, the Spearman correlation coefficient between MMA and ∑As was considerable (r=0.5) since the MMA concentrations were included in the ∑As variable. For these reasons, the authors decided not to include ∑As as an adjustment variable in the MMA multivariate models.

iii) The authors focused the interactions analysis only on nutrients involved in OCM, and others such as Se, ferritin and Mn. Nonetheless, as the reviewer has suggested, we have analysed the maternal tobacco consumption as a possible confounder through two approaches: 1) adjusting the main models with the maternal tobacco consumption during the first trimester of pregnancy and 2) evaluating the smoking habit as a potential modifier by interaction terms. In this analysis, we did not observe any influence on the negative association of MMA concentrations and the MSCA subscales

We have added some discussion about this possible influence between MMA and smoking.

Discussion section, page 21, 1st paragraph: Apart from the influence of some nutrients and elements on As neurotoxicity, the association between prenatal MMA concentrations and the MSCA scales observed in our study could be reflecting the relationship between other variables with an influence on As metabolism, such as maternal smoking and the OCM nutrients. Some studies have shown an association between smoking during pregnancy and OCM nutrients; women smokers presented lower folate and vitamin B12 levels and higher homocysteine levels (Tuenter et al. 2019) than non-smokers. Nevertheless, we have analysed this potential confounder through two approaches: 1) adjusting the main models with the maternal tobacco consumption during the first trimester of pregnancy, and 2) evaluating the smoking habit as a potential modifier by interaction terms. In both approaches, we did not observe any influence of the maternal smoking habit on the inverse association between MMA concentrations and the MSCA subscales (data not shown). However, due to the complexity of the interrelations between As metabolism, micronutrients and other factors, such as genetic factors, the interpretation of the association of MMA concentrations and child neurodevelopment should be taken with caution.

2. The process of selecting covariates seem thoroughly described, but it is complex and hard to keep track of. Have the authors considered using DAGs? Then fix the covariates between models, at least keep to the same covariates among all the models of inorganic arsenic.

Regarding the process of selecting covariables, first we performed a thorough review of the literature about this topic. Then, we selected the covariates through the statistical analysis described in the manuscript. We thank the reviewer for his/her suggestion about the selection of variables. However, we

think that each MSCA subscale represents a different aspect of neuropsychological development which could be influenced by different maternal and child characteristics. In addition, each exposure variable could also be associated with different habits or sources (for instance AB comes mainly from fish consumption and inorganic As comes mainly from consumption of rice and other cereals). So, the authors consider that the statistical analysis will be more accurate if we do not fix the same covariates for all the multivariate models.

Although we did not use DAGs for covariate selection, we accounted for the causal relationships between them before analysis. In the two steps process, we firstly selected those covariates that better predicted the outcome in order to minimize random residual error, but also avoided adjusting for variables that could mediate in the relationship between exposure and outcome. In the second step, we adjusted for any potential remaining confounder to minimize systematic error. Although DAGs are a good theoretical instrument for variable selection, in practice it is very complex to accurately represent the causal relationships between all the variables involved in the study.

Nevertheless, in order to clarify the information about the process of selection of covariates and make reading easier, we have restructured this paragraph, and we have also created three tables (Tables S4.1, S4.2, S4.3) with the different covariables and confounders for each model (instead of Appendix 2).

3. Results shown in Figure 3 raises concern about multiple testing, and this has not been discussed. Also, this testing of effect modification should be included in the objectives of the study with a clear hypothesis.

We have added the analysis of the effect interaction to the objectives of the study.

Introduction section, page 7, last paragraph: The aim of this study is to explore the relationship between prenatal total concentrations of As (TAs) as well as the different urinary As species (arsenobetaine [AB], DMA, MMA and iAs) and the methylation efficiency in the first trimester of pregnancy and children's neuropsychological development assessed at 4–5 years of age in a Spanish birth cohort. We also studied the effect modification produced by sex, the maternal levels of certain nutrients and elements (serum Mn, Se and ferritin, and urinary Zn), as well as the intake of vitamins (folate and vitamins B_6 and B_{12}) during pregnancy.

Regarding multiple testing in the interaction analysis, we agree with the reviewer in the general risks associated with multiplicity of analysis and it has been recognized in the discussion section of the manuscript:

Discussion section, page 21, 2nd paragraph: In the present work, multiple analyses were performed, particularly in the interaction analysis, therefore results should be taken with caution because some significant associations could result from chance. Coefficients and their confidence intervals should be

taken as a global representation of the pattern of the relations between the variables involved in the study (Rothman 1990).

Reference:

Rohtman KJ. No adjustments are needed for multiple comparisons. Epidemiology. 1990; 1:43-6.

Minor comments

1. Introduction, first paragraph: It would be informative to mention something about organic As and sources of exposure for that already here in the beginning. As this paper covers both inorganic and organic As.

As the reviewer has suggested, we have added some information about sources of exposure to organic forms and their metabolism in the Introduction section.

Introduction section, page 5, 1st paragraph: The route of exposure to the organic forms (oAs), arsenobetaine (AB) and other complex forms of As, such as arsenolipids and arsenosugars, is mainly through seafood and fish consumption (Agence nationale de sécurité sanitaire de l'alimentation de l'environnement et du travail 2011; Agéncia Catalana de Seguretat Alimentaria 2020).

Introduction section, page 5, 2nd paragraph: Regarding the most complex forms of As, AB is excreted without changes through the urine, but arsenosugars and arsenolipids seem to be metabolised, producing DMA (Molin et al. 2012; Taylor et al. 2017).

2. Introduction, end of second paragraph: Please do not mix essential elements and toxic elements such as cadmium in one sentence. It would also be informative to expand a bit about this to understand how essential elements can affect As metabolism, for example via one-carbon metabolism. What about cadmium, how does that affect As metabolism?

As the reviewer has suggested, we have added some of information about the influence of OCM nutrients, and other essential and toxic elements on the As metabolism. We have also separated the essential and the toxic elements.

Introduction section, page 5, 2nd paragraph: Several factors seem to affect methylation efficiency~~,~~ ~~particularly~~ such as the intake of some nutrients and elements. Particularly, evidence has also been found of the influence of nutrients that participated in one-carbon metabolism (OCM), such as vitamins B_6 and B_{12}, and folate, which are involved in the synthesis of S-adenosylmethionine (SAM), the main donor of the methyl group in iAs methylation (Kurzius-Spencer et al. 2017; Laine et al. 2018) . Moreover, other essential elements appear to have an influence on As metabolism, such as manganese (Mn), ~~cadmium (Cd),~~ selenium (Se), or zinc (Zn) (López-Carrillo et al. 2016; Rahman et al. 2019; Trasande et al. 2014; Valeri et al. 2016).

Toxic elements, such as cadmium (Cd) also seem to be related to the efficiency of As metabolism, by binding to reduced glutathione, an antioxidant involved in the reduction process (Nordberg et al. 2005).

3. Introduction, study aim should also contain the effect modification by nutrients as indicated above.

As the reviewer has suggested, we have added the analysis of the effect interaction to the objectives of the study:

Introduction section, page 6, last paragraph: The aim of this study is to explore the relationship between prenatal total concentrations of As (TAs) as well as the different urinary As species (arsenobetaine [AB], DMA, MMA and iAs) and the methylation efficiency in the first trimester of pregnancy and children's neuropsychological development assessed at 4–5 years of age in a Spanish birth cohort. We also studied the effect modification produced by sex, the maternal levels of certain nutrients and elements (serum Mn, Se and ferritin, and urinary Zn), as well as the intake of vitamins (folate and vitamins B_6 and B_{12}) during pregnancy.

4. Page 8, Covariates and potential confounders, line 4: Correct repetitive word "…filled during pregnancy and, and later…"

We thank the reviewer for noticing this mistake. We have removed the repeated word.

5. Page 9, 2nd paragraph: What is plasmatic? Should it not be plasma ferritin?

We thank the reviewer for noticing this mistake. We have corrected the word.

6. Page 10, section of effect modification: How were the cut-off points chosen for Mn, Se, Zn, B6 and B12?

The cut-off points chosen for Mn, Se, Zn, B6 and B12 were selected as explained in the statistical analysis.

Material and methods section, page 11, Statistical analysis, 1st paragraph: Effect modification analysis was performed by creating an interaction term between ~~interacting~~ the exposure variable ~~with~~ and the potential modifier: child's sex, maternal nutrients categorized by the median: Mn (< and ≥ 1.44 µg/L), Se (< and ≥ 79.8 µg/L) and Zn (< and ≥ 363.7 µg/L), intake of vitamins B_6 (< and ≥ 2.3 mg/day) and B_{12} (< and ≥ 9.4 mg/day), ferritin (categorized as iron deficiency [ID] as < and ≥ 15 µg/L), and intake of folate (categorized by the intake recommendation during pregnancy, < and ≥ 600 µg/day].

We selected the median as cut-off points for Mn, Se and Zn because we did not find a clear reference value for these elements. For vitamins B_6 and B_{12} we decided to use the median because we have few cases with intake below the recommendations.

7. Page 15, lines 33-34: "In a Mexican study, the authors found a significant association between urinary MMA concentrations and problem solving…" Please indicate direction of the association.

In the Mexican study cited (Rosado et al., 2007), only the results about the relationship between urinary total As and several neurodevelopment scales were shown. In the paper, the authors only showed whether the association of each As metabolite and the neurodevelopment scales was statistically significant, but the direction and the magnitude of the associations were not stated.

Hence, we do not indicate the direction of the association of the study because, unfortunately, we have not this information.

8. Page 18, lines 16-17: …, and As metabolism efficiency seems to change during pregnancy,…. This is a highly important point, and this increased metabolism starts very early in pregnancy. Could the authors elaborate on time of initiation and support with a reference. For example, Arsenic methylation efficiency increases during the first trimester of pregnancy independent of folate status. Gardner RM, Nermell B, Kippler M, Grandér M, Li L, Ekström EC, Rahman A, Lönnerdal B, Hoque AM, Vahter M.Reprod Toxicol. 2011 Feb;31(2):210-8. doi: 10.1016/j.reprotox.2010.11.002. Epub 2010 Nov 13.PMID: 21078382

The authors thank the reviewer for her/his suggestion. We have added some information about this topic in the Introduction section.

Introduction section, page 5, 2nd paragraph: Another factor that could affect the As metabolism is the pregnancy status. An increase in As methylation has been observed during pregnancy, denoted by a higher %DMA and lower %MMA (Gardner et al. 2011; Hopenhayn et al. 2003). This increase in As methylation efficiency appears more rapidly during the first trimester of pregnancy (Gardner et al. 2011).

ANEXO 3:

Otras publicaciones y trabajos presentados

Artículos publicados como primera autora

Soler-Blasco, R., Murcia, M., Lozano, M., Aguinagalde, X., Iriarte, G., López-Espinosa, M.J., Vioque, J., Íñiguez, C., Ballester, F., y Llop, S. (2019). Exposure to mercury among 9-year-old Spanish children: Associated factors and trend throughout childhood. *Environment International*, 130: 104835; https://doi.org/10.1016/j.envint.2019.05.029

Artículos publicados como coautora

Vioque, J., Garcia-de-la-Hera, M., Gonzales-Palacio, S., Torres-Collado, L., Notario-Barandiaran, L., Oncina-Canovas, A., **Soler-Blasco, R.**, Lozano, M., Beneito, A., y Navarrete-Muñoz, E.M. (2019). Reproducibility and Validity of a Short Food Frequency Questionnaire for Dietary Assessment in Children Aged 7–9 Years in Spain. *Nutrients* 11 (4): 933. https://doi.org/doi:10.3390/nu11040933.

Lozano, M., Murcia, M., **Soler-Blasco, R.**, Iñiguez, C., Irizar, A., Lertxundi, A., Basterrechea, M., Santa Marina, L., Amorós, R., Broberg, K., Ballester, F y Llop S. (2020). Prenatal Se Concentrations and Anthropometry at Birth in the INMA Study (Spain). *Environmental Research*, 108943. https://doi.org/10.1016/j.envres.2019.108943.

Irizar, A., Molinuevo, A., Andiarena, A., Jimeno-Romero, A., San Román, A., Broberg, K., Llop, S., **Soler-Blasco, R.**, Murcia, M., Ballester, F y Lertxund, A. (2021). Prenatal Manganese Serum Levels and Neurodevelopment at 4 Years of Age. *Environmental Research* 197: 111172. https://doi.org/10.1016/j.envres.2021.111172

Lozano, M., Murcia, M., **Soler-Blasco, R.**, Casas, M., Zubero, B., Riutort-Mayol, G., Gil, F., Olmedo, P., Grimalt, JO., Amorós, R., Lertxundi, A., Vrijheir, M., Ballester, F y Llop, S. (2021). Exposure to Metals and Metalloids among Pregnant Women from Spain: Levels and Associated Factors. *Chemosphere* 286: 131809. https://doi.org/10.1016/j.chemosphere.2021.131809.

Lozano, M., Murcia, M., **Soler-Blasco, R.**, González, Ll., Iriarte, G., Rebagliato, M., Lopez-Espinosa, M.J., Esplugues, A., Ballester, F y Llop, S. (2021). Exposure to Mercury among 9-Year-Old Children and Neurobehavioural Function. *Environment International*, 146 (106173). https://doi.org/10.1016/j.envint.2020.106173.

Binter, A.C., Bernard, J.Y., Mon-Williams, M., Andiarena, A., González-Safont, Ll., Vafeiadi, M., Lepeule, J., **Soler-Blasco, R.**, Alonso, L., Kampouri, M., Mceachan, R., Santa-Marina, L., Wright, J., Chatzi, L., Sunyer, J., Philippat, C., Nieuwenhuijsen, M., Vrijheid, M y Guxens,

M. (2022). Urban Environment and Cognitive and Motor Function in Children from Four European Birth Cohorts. *Environment International,* 158 (106933). https://doi.org/10.1016/j.envint.2021.106933.

Lozano, M., Yousefi, P., Broberg, K., **Soler-Blasco, R.**, Miyashita, C., Pesce, G., Jim, W.J., Rahman, M., Bakulski, K.M., Haug, L.S., Ikeda-Araki, A., Huel, G., Park, J., Relton, C., Vrijheid, M., Rifas-Shiman, S., Oken, E., Dou, J.F., Kishi, R., Gutzkow, K.B., Annesi-Maesano, I., Won, S., Hivert, M.F., Fallin, M.D., Vafeiadi, M., Ballester, F., Bustamante, M y Llop, S. (2022). DNA Methylation Changes Associated with Prenatal Mercury Exposure: A Meta-Analysis of Prospective Cohort Studies from PACE Consortium. *Environmental Research,* 204: 112093. https://doi.org/10.1016/j.envres.2021.112093.

Pósters presentados a congresos

Soler Blasco, R., Murcia, M., Carrasco, P., Cases, S., López Espinosa, M.J., Vioque, J., Íñiguez, C., Ballester, F y Llop. (2018). Exposición a mercurio en niños/as de 9 años de valencia. Evolución de niveles y factores asociados. *XXXVI Reunión Anual de la Sociedad Española de Epidemiología (SEE) y XIII Congresso da Associação Portuguesa de Epidemiologia (APE)*. Lisboa, Portugal, septiembre, 2018.

Soler Blasco, R., Murcia, M., Ballester, F., Lozano, M., González Safont, Ll., Ibarluzea, J., Irizar, A., Lertxundi, N., Santa Marina, L y Llop, S. Prenatal manganese exposure and neurodevelopmental effects at 1 year of age in the INMA cohort (Spain). *31st annual conference of the International Society for Environmental Epidemiology*. Utrecht, Países Bajos, agosto 2019.

Soler Blasco, R., Murcia, M., Lozano, M., Malaguarnera, M., González-Safont, Ll., López Espinosa, M.J., Esplugues, A., Ballester, F y Llop, S. Prenatal exposure to total arsenic and its association with neuropsychological development at 5 years old in a Spanish cohort. *The 8th international congress and exhibition on arsenic in the environment.* Congreso virtual, Países Bajos, junio 2021.

Soler Blasco, R., Murcia, M., Lozano, M., Lertxundi, A., Irizar, A., Lertxundi, N., Santa-Marina, L., Signes, A., Imaz, L., Ballester, F y Llop, S. Maternal arsenic species and methylation capacity: concentrations and associated factors in the Spanish INMA cohort. *The 8th international congress and exhibition on arsenic in the environment.* Congreso virtual, Países Bajos, junio 2021.

Comunicaciones orales presentadas a congresos

Soler Blasco, R., Murcia, M., Irizar, A., Zubero, B., Ballester, F y Llop, S. Niveles de manganeso durante el embarazo y factores asociados a la exposición. Proyecto INMA. *XV Congreso de Salud Ambiental y V Congreso Iberoamericano de Salud Ambiental.* Valencia, España, mayo, 2019.

Soler Blasco, R., Murcia, M., Lozano, M., Olmedo, P., González, Ll., López- Espinosa, M.J., Rebagliato, M., Ballester, F y Llop, S. Exposición a arsénico durante el embarazo: niveles en orina y factores asociados. *XXXVII Reunión Anual de la Sociedad Española de Epidemiología (SEE) y XIV Congresso da Associação Portuguesa de Epidemiologia (APE).* Oviedo, España, septiembre 2019.

Soler Blasco, R., Murcia, M., Lozano, M., Irizar, A., Lertxundi, N., Imaz, L., Santa-Marina, L., Ballester, F y Llop, S. Especiación de arsénico durante el embarazo: niveles y factores asociados en la cohorte INMA. *I Congreso Virtual de la Sociedad Española de Epidemiología, junto a la Associação Portuguesa de Epidemiologia.* Congreso virtual, España. Octubre, 2020.

Soler Blasco, R., Murcia, M., Lozano, M., Sarzo, B., Esplugues, A., Riutort Mayol, G., Vioque, J., Lertxundi, N., Santa Marina, L., Lertxundi, A., Irizar, A., Braeuer, S., Ballester, F y Llop, S. Prenatal arsenic exposure, arsenic methylation efficiency, and neuropsychological development among pre school children in the Valencia and Gipuzkoa INMA cohorts. *17th INMA- Infancia y Medio Ambiente (Environment and Childhood project) Scientific Conference* 2021. Gipuzkoa, España, noviembre 2021.